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THE BOTANICAL REVIEW

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PHYLOGENETIC PRINCIPLES OF THE SYSTEM OF HIGHER PLANTS[1,2]

A. L. TAKHTAJAN[3]
Leningrad University

INTRODUCTION

Construction of the phylogenetic system of the higher plants, just as of the entire plant world, has been and remains one of the most important problems of evolutionary botany. The significance of this problem is not merely theoretical; it is practical in the broad sense of the word. In particular, the setting up of a phylogenetic system constitutes a necessary condition for an evolutionary exposition of the principles of plant classification.

In recent decades many systems have been proposed. They differ a great deal and frequently are based on diametrically opposite principles. They differ also in the degree of elaboration and detailed information, as well as in the methods of graphic presentation of the phyletic connections. Development of new phylogenetic systems does not represent in itself, however, a progressive approximation to the true phyletic correlations of organisms. Phylogeny proceeds in zigzags, frequently regressing, sometimes reviving obsolete opinions, and even returning to stages already attained in the early development of the science. The voluminous book by the venerable American botanist, Campbell (1940), may serve as a typical and characteristic example of such regression in phylogeny. This book represents, in many respects, a true anachronism. In it the most important achievements of contemporary phylogeny and evolutionary morphology of the higher plants, even including accomplishments of American authors, have been sur-

[1] First published in Russian in Botanicheskii Zhurnal, Vol. 35, 1950.
[2] Authorized English edition translated by Dr. D. I. Lalkow, Sessional Lecturer in Russian, and edited by W. I. Illman, Assistant Professor of Botany, Carleton College, Ottawa, Ont.
[3] Author's transliteration of his name; methods in use in the United States would render it Takhtadzhian or Takhtadzhyan.

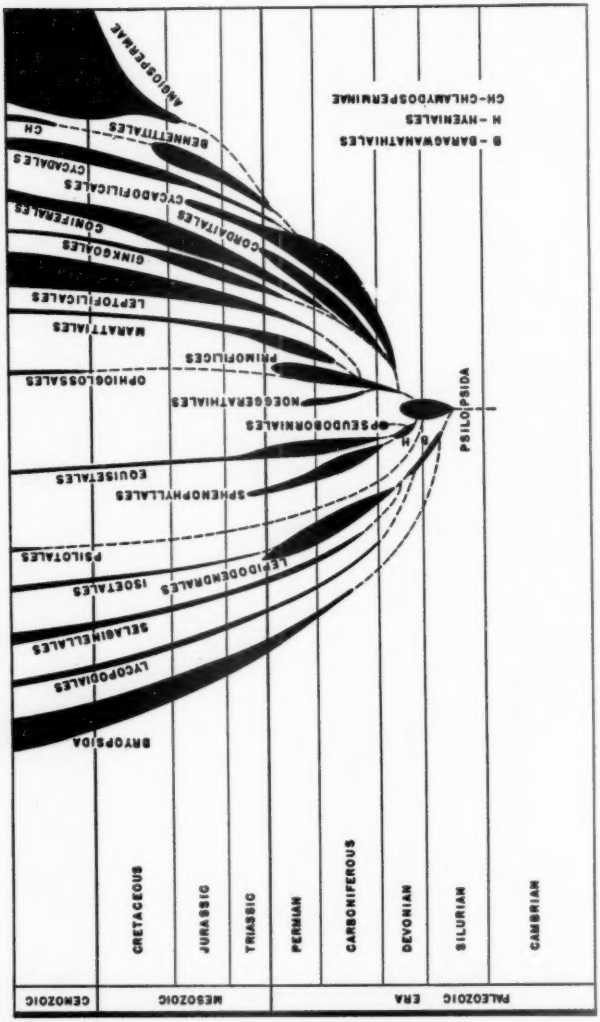

FIG. 1. The geochronological genealogy of the higher plants.

prisingly ignored. We could cite many examples of this kind, regretfully. These examples are excellent proof of the view that the success of phylogenetic investigations depends, not only on knowledge of the actual material but also, in no lesser degree, on the theoretical views on the course and laws of phylogenesis. Knowledge of the regularities of phylogeny constitutes a definite condition for phylogenetic constructions. In this field, beginning with the work of Pavel Horaninov during the 1830's on the phylogeny of plants, Russian science has achieved a great deal through the work of such noted scientists as Vladimir Kovalevski, K. A. Timiriazev and A. N. Severtzov. Professor Christopher Gobi (1916) of Petrograd University published a new and original phylogenetic system of the whole vegetable world. During recent decades research has expanded on a greater scale through the works of B. N. Kozo-Polianskii and a score of younger botanists who have examined questions of morphology and phylogeny in the light of Marxist dialectics.

The purpose of phylogeny is the construction of a genealogical tree of organisms, its substantiation and interpretation. Phylogenetic schemes, apart from the manner of their construction, indicate, in the main, the lines of relationship and the modes of ramification (Fig. 1). Usually, however, these lines of relationship symbolize gradual and uniform transformations of some forms into other forms. There can be nothing more detrimental as far as phylogeny is concerned than this idea of even and gradual evolution. V. I. Lenin wrote that " life and development in nature contains in itself both slow evolution as well as rapid leaps and bounds,—interruptions in continuity ". Evolution is being interrupted in all its modi. Thus, with the idea of a twofold development, we like to visualize the process of phylogenesis as a leap-like transformation of organic forms—change-creating gaps, interruptions in continuity. Therefore taxonomic units appear as nodal points in the development of living nature, links in the continuous but discrete chain. It is precisely the chain, and not the line, that is the symbol of phyletic connections; transitional forms are intermediate links separated by a hiatus both from the ancestral forms and from the descendants. Therefore any philosophy on phylogeny must be based on the idea of discontinuity of evolution. This idea is, however, also of great value in the practice of phylogenetic constructions.

In the present article I do not intend to deal with the task of elaborating on the principles and methods of phylogenetic constructions. My objective is to state and substantiate a phylogenetic system of higher plants (see Appendix I). With the exception of the Bryales, the system has been brought down to families. It embraces all the higher, or telomous plants, apart from the Angiospermae, to which I propose to devote a special work[4].

CONCEPT OF THE TELOME

I propose a new name, "Telomophyta" (telomous plants), to designate the higher plants. The telome is one of the most important constituent elements of the sporophyte in all higher plants, including the bryopsids. At the same time, the name "Telomophyta" emphasizes the main distinction between the higher telomous plants and the lower thallose forms.

Telomes (Fig. 2) are ultimate branchlets of dichotomously branched axial organs in primitive leafless higher plants. The sections of the body connecting them are called "mesomes". The branch bearing a sporangium represents a fertile telome. In their original form telomes and mesomes are expressed in psilophytes. The sporophyte of psilophytes represents a whole system of telomes and mesomes (a polytelome in the terminology of Kozo-Polianskii, 1937). The sporophyte of the Bryopsida, however, represents a single body (monotelome). Organs such as the leaves of modern ferns represent whole systems of intergrown telomes, or syntelomes. The telomes of Zimmermann (1930) and Bower (1935) correspond to the caulodies of Lignier (1903, 1908) and Bertrand (1947) and to the caulosomes of Potonie (1912) and Kozo-Polianskii (1922). The origin of telomous plants was a result of increasing dichotomization and vascularization of the thallose body of their algal ancestors. The sporophyte of psilophytes is the dichotomized and vascularized thallus. In the dichotomization and differentiation of the thallus, polarity was expressed from the very inception. Diversification of the thallus ought to have begun from dichotomization of the upper and lower poles of the plant. In the process of dichotomization of the aerial part of the sporophyte, there was an aggregation of the tetraspores, originally scattered, in the distal portions of terminal branches (telomes) with differen-

[4] The author's book—The System of Angiosperms—is now in press.

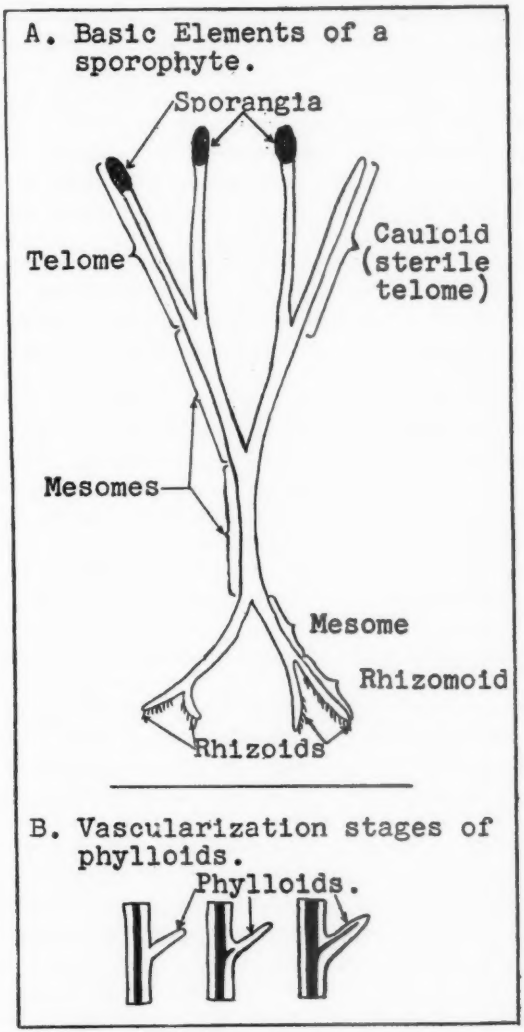

FIG. 2. Sporophyte parts.

tion of these portions into separate organs (sporangia). Inasmuch as sporangia represent, in themselves, modified distal parts of spore-producing branches, their original location was terminal. In this way the terminal branches (telomes) of the aerial part of the sporophyte of primitive plants were initially fertile, with sterile portions connecting them, i.e., mesomes. The inferior pole of the sporophyte was, however, initially sterile. As a result of dichotomization here, telomes did not develop, but instead rhizome-like branches joined by mesomes, and these I propose to name "rhizomoids". Consequently telomes and rhizomoids, together with portions (mesomes) connecting them, were the primary constructive elements of the sporophyte of higher plants. Rhizoids (outgrowths of trichomous origin) probably developed primarily on the rhizomoids proper.

On the aerial part of the plant there developed, early in the process of evolution, vegetative terminal ramuli (sterile telomes), i.e., cauloids (named by Zimmermann "phylloids"). These were formed as a result of sterilization of some of the fertile telomes. On cauloids and mesomes, leaf-like (phylloidal) outgrowths (enations of the type of emergents) sometimes developed. These should best be called "phylloids" (Lignier's term). Finally syntelomes developed as a result of a certain amount of coalescence in the system of telomes.

In evolutionary respects the telome constitutes the most important element of the sporophyte in higher plants. Development of telomes was the most significant evolutionary upgrade (an aromorphosis in the terminology of Severtzov) in the history of the plant kingdom. The name "telomous plant" is thus most applicable to the higher plants. From this point of view, the name "Cormophyta", proposed by Unger, is much less suitable. The term "sprouting plants" (κορμος—trunk, log) is definitely inappropriate for the majority of psilophytes and all bryophytes. By analogous deliberations, "Embryophyta" as well as "Tracheophyta", "Xylophyta", "Stelophyta" and certain other terms proposed during recent decades are wholly inadmissible.

The system of telomous plants begins with the psilophytes. Inherent in this vast group were those chief tendencies of evolution which brought about development of the various phyla of higher plants. The Psilopsida represent that primary phylogenetic plexus

in which the primary links of evolution in the higher plants—bryopsids, lycopsids, tmesopsids (Psilotales), sphenopsids and pteropsids—intermingle. The basic lines of adaptational development of the higher plants, so differing in their terminal ramifications, ascend historically from this primitive alga-like group of ancient land flora.

Originally the dichotomously ramified sporophyte of primitive psilophytes poorly met the demands of subaerial existence. The Bryopsida, the evolution of which centered on the gametophyte, originated in the regressive line of evolution of the sporophyte of psilopsids. However, in this line of development of higher plants we do not observe any noticeable aromorphoses, and evolution here appears purely of a narrow adaptive nature without evolutionary upgrade (an idioadaptive evolution in the terminology of Severtzov). In this case the standard of organization was not increasing. The future of the vegetable world necessitated a progressive evolution of the sporophyte. The sporophyte of a psilophyte could not, however, develop and grow in size successfully without increasing its photosynthesizing surface. For this purpose there existed only two biologically possible ways: either special photosynthesizing organs could develop on the axial portions as out-growths, or else lateral ramuli or entire branch systems could transform into such organs. Nature chose both ways. Firstly, phylloids formed—the " enation leaves " of Lycopsida. Secondly, by cladodification, telomic leaves developed—the leaves of all other phyla of higher plants. In the latter cases, an alternate (Tmesopsida) or a whorled (Sphenopsida) development of minor lateral branch systems occurred, or cladodification of more or less large branch systems took place (Pteropsida).

BRYOPSIDA

The Bryopsida are probably direct descendants of psilophytes (vide Kidston and Lang, 1917; Scott, 1923, 1929; Halle, 1936; Kozo-Polianskii, 1941, 1949; Haskell, 1949). All available data of paleobotany favours, in the main, this assumption. Sporangia of the genera *Horneophyton* (*Hornea*) and *Sporogonites* are of particular interest in this respect. By the presence of a columella (central axis) they strongly suggest the sporangia (capsule) of such bryopsids as *Anthoceros, Sphagnum* and *Andreaea.* The

great resemblance between sporophytes of Anthocerotaceae and *Horneophyton* has been emphasized also by Campbell (1940), although he deduces the psilophytes from the Anthocerotaceae, i.e., the more primitive forms were evolved, according to him, from the simplified descendents. The sporophyte (sporogonium) of the Bryopsida appears to be the result of reduction of the more highly developed sporophyte of the *Horneophyton* type. Simplification of the sporophyte of bryopsids is particularly strongly reflected in its conducting system which became subject to atrophy. Characteristics of progressive development were observed in the sporophyte of bryopsids only in the sporangium, specifically in its wall which shows in many of the highly developed forms fairly complex apparati for dehiscence and dispersion of spores. In all the representatives of bryopsids the sporophyte represents a simple fertile telome, i.e., a single sporangium, situated at the apex of a simple unbranched stalk. The origin of single telomes of bryopsids from dichotomously branched sporophytes or psilophytes is evidenced also by data on teratology (cases of bifurcated or even branched sporophytes in various representatives of Bryopsida). Interestingly, various stages of bifurcation of sporangia have been observed, for example, in *Bryum argenteum* which suggests an analogous phenomenon in the psilophytic genus *Horneophyton*.

The reduction of the sporophyte of bryopsids manifested itself also in the disappearance of the conducting system typical of vascular higher plants. The presence, in many cases, of rudimentary stomata with a structure typical of higher plants appears to be clear evidence of reduction in the conducting (also photosynthesizing) system. This disappearance of specialized conducting tissues in the sporophyte of the Bryopsida is not surprising when we note that even among psilophytes there were reduced forms devoid of conducting tissues. In *Horneophyton* the conducting system disappeared only in the rhizomoid, while in *Sporogonites* it had obviously vanished completely. The latter genus, besides, is closely related to the Bryopsida by the structure of its sporangium. It is likely that *Sporogonites* actually represents the intermediate link between the Psilopsida and the Bryopsida.

The Bryopsida are divided into three classes: Hepaticae, Anthocerotae, Musci. Transitional forms between them are unknown. The evolution of these three branches proceeded along different

courses, but at the source of their genealogy, Bryopsida undoubtedly can be traced back to their common ancestral form. In the evolution of Bryopsida, from forms with upright radially symmetrical gametophytes there originated forms with dorsiventral thallose gametophytes. Thallose forms amidst bryopsids are undoubtedly of secondary origin and developed from radially symmetrical "leafy" ancestors (vide Wettstein 1903, 1908; Golenkin, 1904; Church, 1919; Evans, 1939). Therefore in the class Hepaticae the most primitive group should be considered the order Calobryales; more advanced is the order Jungermanniales; and most advanced are Marchantiales and Sphaerocarpales. The Marchantiales originated from the Jungermanniaceae, to which they are related through the family Monocleaceae. The order Sphaerocarpales, however, is the most simplified among Hepaticae. Class Anthocerotae is phylogenetically deeply enrooted in the Paleozoic era and has, with the rest of Bryopsida, common ancestors among the Psilopsida. In the sporophyte of Anthocerotae certain pure psilophytic traits may still be discerned, although the gametophyte is greatly simplified in them. The genera of the Anthocerotae form a series of reduction in which the most primitive are *Anthoceros* and *Megaceros,* while the most reduced are species of *Notothylas.* As far as class Musci is concerned, it has probably originated from some extinct "leafy" form in Jungermanniales. The Sphagnales are comparatively the most primitive among Musci. Sporophytes of Sphagnaceae possess definite characteristics of resemblance, not only with sporophytes of such a form as *Anthoceros,* but even with sporophytes of certain Psilopsida, e.g., *Horneophyton.* In the structure and development of *Sphagnum* there become apparent a score of characteristics which indicate relationship, on the one hand, with Hepaticae and Anthocerotaceae, and, on the other, with the rest of Musci. The Andreaeales represents in some respects, so to speak, an intermediate group between the Sphagnales and Bryales. The vast order Bryales appears to be the most advanced among Bryopsida.

LYCOPSIDA

The Lycopsida represent an entirely separate (microphyllous) line of evolution of higher plants, being characterized by the absence of true (telomous) leaves, which are replaced in them by

phylloids. The phylloids of lycopsids are of emergent origin, as a rule simple, more rarely bifurcate or very seldom thrice dichotomized (Devonian genus *Colpodexylon*). The absence of gaps in the stele for phylloid traces and their presence for branches is characteristic. The position of the solitary sporangia of the Lycopsida in axils of phylloids or on the stem above axils, or very seldom on the adaxial side of phylloids, is also very interesting. Sporangia on phylloids are always borne singly. The Lycopsida originated directly from psilophytes. The very presence of such microphyllous psilophytes with terminal position of sporangia, as *Asteroxylon,* witnesses to the fact that among a certain part of Psilopsida there was also in evidence a phylloidal line of evolution, in which the organs of photosynthesis occurred in the shape of emergent outgrowths (enations) on the axial organs. Thus it follows that lycopsids might have developed directly from psilopsids. In accepting the Psilopsida with their terminal position of sporangia as the primary type of higher plants, it is an easy matter to deduce from them the Lycopsida with the lateral or axillary position, typical of them, if one were to admit a development by way of overtopping (dichopodial development) of the whole system of ramification of a sporophyte, as well as shortening of lateral sporangia-bearing branchlets. Thus it is quite possible to assume that in the " microphyllous " forms of psilophytes, along with overtopping and increase in growth of the main or central axis, there developed a gradual decrease and simplification of the lateral branchlets so that they became reduced to short simple fertile telomes. As a result of reduction of the stalks of these telomes, sporangia appeared to be ultimately distributed between the phylloids on the main or central axis. In this manner, in lycopsids the position of sporangia appeared to be, so to speak, pseudo-lateral, for as a result of reduction and subsequent more or less complete disappearance of the axial part of the fertile telomes, sporangia changed from their terminal position to the lateral. Originally the lateral sporangia of the Lycopsida were located intermittently between the sterile enations, but subsequently they migrated into their axils and thus were under better nutritional and protective conditions.

An entirely different picture on the origin of the Lycopsida type of sporangium position is proposed by Zimmermann (1930, 1938)

and Lam (1948). They also assume the primitive character of terminal position of sporangia of psilophyte ancestry, although they interpret the axillary position of sporangia of lycopsids in a different manner entirely. In the opinion of these authors, the photosynthetic organs of lycopsids appear to be, not enations on the surface of the axial organs, but sterilized and reduced telomes (telomic leaves). In the opinion of Lam (1948), the origin of leaves of lycopsids from reduced telomic leaves was confirmed by the forked tips of leaves in the genus *Protolepidodendron* and by the presence of two parallel veins at the bases of leaves in *Protolepidodendron, Drepanophycus, Sigillaria* and *Pleuromeia*. The fact, however, is that phylloids of lycopsids, as the phylloids in *Asteroxylon*, were primarily sterile and thus were never telomes. A telome is primarily an element of the aerial part of the sporophyte and was initially spore-producing. Phylloids, however, are secondary and formed on the surface of telomes and on their derivatives, and were initially sterile. Only later did fertile phylloids develop from sterile ones by sporangia migrating into their axils. The axillary position of sporangia in the majority of Lycopsida does not prove unity in origin of microphyllum and sporangium. Phylloids of the Lycopsida, just as phylloids on *Asteroxylon*, developed entirely independently from sporangia. Phylloids may be completely devoid of sporangia; and sporangia, in turn, may have no connection whatever with phylloids. From this point of view the question of sporangium position in the Siluro-Devonian genera is of great interest (*Zosterophyllum* and *Bucherta*). Sporangia in these plants sat terminally on short and simple lateral branchlets. Thus we have before us a transition type from terminal position of sporangia in the psilophytes towards their lateral position on the stem in the most primitive Lycopsida. These two genera, therefore, indicate that transition forms between the main types of distribution of sporangia are perfectly real. Further evolution in the sporangium position in Lycopodiales proceeded from the lateral (cauline) type, through the axillary, towards the adaxial (on the upper side of the phylloid). In this way the emergent organs of photosynthesis in Lycopsida, in distinction from telomic foliage of other types of higher plants, were initially sterile and in many representatives remained so. Therefore sporophylls of lycopsids are non-homologous with telomous and initially fertile sporophylls of Sphenopsida

and Pteropsida. Lam (1948) proposed to name such fertile sporophylls " stegophylls ", which term can not be recognized as conclusively suitable. We suggest naming them " sporophylloids ".

The early lycopsids, similar to their ancestors, the psilophytes, were minute, herbaceous and homosporous plants. The most primitive group of Lycopsida was the Baragwanathiales, this being the connecting link between the microphyllous psilophytes and typical lycopsids. From the order Baragwanathiales arose, on one hand, the homosporous and eligulate Lycopodiales, and, on the other hand, heterosporous and ligulate Selaginellales and Lepidodendrales. Lycopodiaceae are more primitive in certain of their characteristics than the Devonian *Protolepidodendron*. Therefore lycopods may not be considered as direct descendants of Protolepidodendraceae, although they are undoubtedly of common origin. Orders Selaginellales and Lepidodendrales are no doubt of common origin, but their divergency occurred very early, i.e., in the Devonian period. But while the Lepidodendrales became woody plants, the representatives of the Selaginellales remained permanently as herbs. The order Selaginellales is composed of two families—Selaginellaceae and Miadesmiaceae. The megasporangium in the Carboniferous genus *Miadesmia* was completely occluded in the convoluted sporophylloid, provided with many lobes and outgrowths. This covering was compared by some authors with the integument of seed plants, although as far as its origin is concerned, it has nothing in common with the latter. An analogous covering from a sporophylloid was also developed in *Lepidocarpon* (ex Lepidodendrales), sometimes segregated and classified, for no conclusive reasons, as belonging to a special order, Lepidocarpales. The family Lepidocarpaceae represents a higher stage of the morphological evolution of the Lepidodendrales. Woody Lycopsida were not distinguished by sufficient plasticity and in the course of further evolution diminished in importance and became extinct during the Triassic. The ancient herbaceous forms, however, such as *Lycopodium* and *Selaginella*, proved to be more tenacious, and have persisted to the present. Among the family of the woody *Lepidodendron* and *Sigillaria* only such a secondary herbaceous form as *Isoetes* survived. This genus appears to be the last descendant of the gigantic *Lepidodendron–Sigillaria* complex of the Carboniferous era. The origin of *Isoetes* and in

general the evolution of the Isoetales present considerable interest. The families Pleuromeiaceae and Isoetaceae are placed in this order. Seward (1910) pointed out the kinship of the contemporaneous genus *Isoetes* with the fossil Triassic genus *Pleuromeia.* Further investigations only confirmed this hypothesis (Mägdefrau, 1932; Hirmer, 1933). These genera correspond in structure of stem and root, and both are provided with ligular phylloids and trabecular sporangia. On the other hand, however, *Pleuromeia* has much in common with the genus *Sigillaria.* Thus *Pleuromeia* appears to be a connecting link between *Sigillaria* and the Isoetaceae, though it is more closely related to the latter. For a long period it was assumed that sporangia of *Pleuromeia* are located, in distinction to all the remaining lycopsids, on the abaxial side of their sporophylloids. Hirmer (1933) has even drawn up a scheme which is supposed to clarify such quasi abaxial position of sporangia in *Pleuromeia.* In his opinion the original type of sporophylloids (sporophylls) of lycopsids was the peltate type bearing two sporangia, one on its upper and one on its lower side (adaxial and abaxial respectively). In the case of a reduced adaxial sporangium there formed sporophylls of the *Pleuromeia* type, whereas if the abaxial sporangium disappeared, there developed a sporangium position common to remaining Lycopsida. Hirmer's hypothesis can not stand the test, however, since peltate sporophylls are absolutely unknown among fossil or contemporary Lycopsida. The fact is that sporangia of lycopsids were not situated on phylloids, which were initially sterile enations, but actually on stems between phylloids. Hirmer's hypothesis is therefore purely speculative and perfectly inconclusive. The question arises, then, whether the position of sporangia in *Pleuromeia* is actually abaxial. In comparing *Pleuromeia* with the contemporary genus *Isoetes* we are enabled to solve this question in the negative. The sporangia of *Isoetes* are so deeply sunken in the phylloid that there remains only a thin layer of tissue on the abaxial side of the latter; and thus, as Eames correctly points out, the position of the sporangium in the fossil state may appear abaxial. In Eames' opinion the seemingly abaxial distribution of sporangia in *Pleuromeia* may be explained precisely by the fact that the sporangium is embedded still deeper in the phylloid's tissue and dorsally there remains therefore a very tender and thin layer of cells. This is why sporophylloids in

Pleuromeia seem to be perfectly identical on both upper and lower sides, which is particularly conspicuous by the absence of the median vein. An adaxial position of sporangia in *Pleuromeia* was also considered as probable by the paleobotanist Walton (1940). Illuminating the origin of the contemporary *Isoetes,* the lower Cretaceous genus *Nathorstiana* occupies, so to speak, an intermediate position between *Pleuromeia* and *Isoetes.* The genus *Isoetes* appears to be, according to all its characteristics, the ultimate link in the series of forms which are characterized by increasing reduction in size of plants and by simplification of rhizophores (stigmarian axis). *Isoetes* resembles a reduced form of *Nathorstiana,* and the latter appears undoubtedly to be derived from a reduced type of *Pleuromeia.* All this regressive series begins with the genus *Sigillaria* and terminates with *Isoetes.* In the contemporary genus *Isoetes* there is observed very distinctive multiciliate sperms which by their structure and the distribution of cilia differ sharply from the multiciliate sperms of the Equisetaceae and Polypodiaceae. Spermatozoids of *Isoetes* originated from biciliate sperms of the type of sperms in *Lycopodium.* It is interesting to note, however, that in *Lycopodium* with biciliate sperms, triciliate ones also occur, which probably could have brought about the appearance of multiciliate species in other lines of evolution.

TMESOPSIDA

Phylum Tmesopsida is represented at present by two genera, *Psilotum* and *Tmesipteris* (family Psilotaceae). Having thoroughly compared Psilotaceae and Psilophytaceae, Kidston and Land (1923) arrived at the conclusion that the sporangiophores of *Psilotum* and *Tmesipteris* represent in appearance axial structures favourably comparable with the sporangia-bearing branches of the Psilophytales. The investigation of anomalous branched sporangiophores carried out by B. Sahni (1923–1925) showed that the latter developed from repeatedly dichotomized branches. This conclusion, however, may be extended also to the vegetative leaves of Psilotaceae. Fertile and vegetative foliar organs of Psilotaceae are undoubtedly homologous. This may be seen, first of all, in the transitional stages observed frequently between the sterile leaves and the sporophylls (Solms-Laubach, 1884). In addition, the sterile and fertile leaves on thin branches are allied with one an-

other by their phyllotaxy, which further proves their homology (Goebel, 1930; Schoute, 1938). Both sterile and fertile leaves of Psilotaceae are of telomous origin; they are specialized syntelomes. In *Psilotum*, however, as I reported in 1943, the syntelome became reduced to a single simple telome. Lam (1948) expressed a similar point of view on the origin of the leaves of Psilotaceae. According to Lam, the leaves of *Tmesipteris* are probably syntelomous, while the leaves of *Psilotum* are monotelomous. The hypothesis of the telomous origin of foliar organs in Psilotaceae throws light on the origin of their singularly distributed sporangia. The sporophylls of both genera are forked but have a synangium on their upper side. Sometimes, as an anomaly, they are repeatedly dichotomized. This phenomenon may be regarded as atavism. The synangium position in the Psilotaceae represents a modification of the terminal position of free sporangia in their psilophytous ancestors. A branch bearing a terminal synangium developed as a result of fusion between sporangia and their stalks. The sporophyll in the Psilotaceae represents a syntelome, the lateral branchlets of which are sterile, whereas the central branchlet is fertile. What then is the position of the Psilotaceae in the system of higher plants? The family has been grouped both with the Lycopsida and with the Sphenopsida. Most frequently Psilotaceae were compared and associated with Lycopodiaceae. This association, however, is unnatural and inconclusive for the very reason that the Psilotaceae, as distinguished from the Lycopodiaceae, are entirely devoid of roots, and that their foliar organs are of telome origin, their sporangia coalesced into synangia with a completely different position. Moreover, their antheridia are superficial, not sunken; and finally their spermatozoids are of a different structure. Psilotaceae differ rather strongly also from Sphenopsida, although, judging by the morphological nature of their foliar organs, the former obviously stand closer to the latter than to the Lycopodiaceae. In the opinion of many contemporary botanists, Psilotaceae are direct descendants of the Psilophytales (Kidston and Lang, 1917, 1921; B. Sahni, 1925; Zimmermann, 1926, 1930, 1938; Eames, 1936; Kozo-Polianskii, 1949; and others). On the strength of these assumptions, however, the Psilotales and Psilophytales can not be combined into a single subdivision, as Eames (1936), Darrah (1939) and Lam (1948) assume. Psilotaceae are not related more

closely with the Psilophytales than is *Anthoceros,* for example, while among the most primitive fossil Lycopsida, Sphenopsida and even Pteropsida, there were forms which could be much more closely associated with the psilophytes. If these subdivisions are more closely associated with the Psilophytales becaues of their most ancient fossil forms, there should be even more reason to combine the psilophytes with any of these groups than with the Psilotaceae. In view of this, the Psilotaceae—being known descendants of the psilophytes, similarly to lycopods, horsetails and ferns—should be considered as a separate phylum (subdivision) of telomous plants. This subdivision was named " Tmesopsida " by the author (1941).

SPHENOPSIDA

At the time that Jeffrey (1899, 1902) reclassified all " vascular " plants into two phyla—Lycopsida and Pteropsida—he referred to the Lycopsida not only the Lycopodiaceae, but also the Psilotaceae, Sphenophyllaceae, Calamitaceae and Equisetaceae. The group Lycopsida, however, in Jeffrey's conception was very heterogeneous and unnatural. From a contemporary viewpoint, sphenopsids represent, in a broad sense, an entirely independent line of evolution of higher plants. Paleobotanist D. H. Scott (1909) was the first to speak in favour of segregating the sphenopsids from the lycopsids into a separate subdivision, Sphenopsida. He was motivated by the fact that the Sphenopsida are relatively megaphyllous (i.e., by a different nature of foliar organs) and are " sporangiophorous " plants (i.e., have their sporangia on special appendages, or sporangiophores). Scott included, however, the Psilotaceae in the group Sphenopsida. This was an error on his part, though a lesser one than inclusion of the Psilotaceae in the group Lycopsida. Finally, according to more recent authors, such as Zimmermann (1930, 1938) and Eames (1936), the Sphenopsida represent a group of higher plants entirely separate and free from foreign elements.

One of the most characteristic traits in the Sphenopsida is their small whorled leaves. In Jeffrey's opinion, the leaves of Sphenophyllales are microphyllous, analogous to the leaves in *Lycopodium,* for example. Lignier (1903, 1908) considered the foliar organs of the Equisetales as cauloids, not phylloids, and deduced them from

the leaves of microphyllous primofilices. The leaves of sphenopsids are not of enational but of telomous origin. However, similarly to the leaves of the Tmesopsida and in contrast to those of megaphyllous pteropsids, they did not develop from major branch systems but from minor ones (Arber, 1921; Benson, 1921; Eames, 1936). The leaves of Tmesopsida and Sphenopsida are meiophyllous (Benson's terminology), not megaphyllous. While the leaves of Tmesopsida, however, have always been distributed spirally, those of the Sphenopsida developed from lateral branches which tended to have a whorled arrangement.

The evolution of Sphenopsida began from the very primitive Devonian group, Hyeniales. The Hyeniales originated among Psilophytales of the *Psilophyton* type, the sporangia of which were borne terminally on recurved, shortly bifurcated branchlets. The order Hyeniales appears, in the true sense of the word, an intermediate link between psilophytes and typical sphenopsids. The phylogenetic value of the Hyeniales is great. Here originated not only such primitive forms as the Upper Devonian order Pseudoborniales, but even more highly organized plants, such as the Sphenophyllales and *Asterocalamites*. The characteristics of the Hyeniales, e.g., the structure and position of their sporangia, give us a clue for comprehending more complex structures of recent forms. Order Sphenophyllales appears to be a side branch in the evolution of Sphenopsida. Each sporophyll in Sphenophyllaceae usually consisted of two lobes, adaxial (superior) and abaxial (inferior); the former was fertile, the latter usually sterile, though occasionally both were fertile (*S. fertile*). In the latter case, analogy and relationship with the Hyeniales manifested itself graphically. The sporangium position in them is, as a rule, still obviously terminal. In those cases where the adaxial lobe is reduced, the sporangia may be found on an axillary sporangiophore. In some species the stalk of the sporangiophore (remnant of the adaxial segment of the sporophyll) is completely reduced, and sporangia prove to be sessile on the upper side of the inferior lobe. All these modifications have an adaptive character which contributes to a better protection of the sporangia. When, however, anatropous sporangia are sessile on the interior side of peltate expansions, which, in approximating one another peripherally, form a compact strobilus, then such protection proves most effective. The sporo-

phylls in the genus *Cheirostrobus,* most closely related to *Sphenophyllum,* became most complex. The structure of strobili, also, is fairly complicated in the genus *Tristachya,* as described by the Polish botanist J. Lilpop (1937). He segregated it, on no sound grounds, into an independent group, Tristachyineae, which has since been given ordinal rank by Emberger (1945).

The order Equisetales also originated directly from the Hyeniales. The most primitive and ancient family appears to be Asterocalamitaceae, of which the leaves were cloven dichotomously into filiform segments, even though the sporophylls were peltate, as they are, for example, in a contemporary form of *Equisetum.* In comparing this family with the Hyeniales we were able to deduce that the sporophylls of Asterocalamitaceae, as distinguished from sporangiophores of the genus *Sphenophyllum,* are homologous with the entire leaf and resulted from expansion and coalescence of terminal segments of the sporangia-bearing branch system of ancestral forms (Zimmermann, 1930, 1938). The families Calamitaceae and Equisetaceae are more advanced. The modern family Equisetaceae differs from Calamitaceae in that its stems are devoid of secondary tissues, and that in its strobili there are no whorls of sterile leaves alternating with sporophylls. Equiseta appear to be the result of reduction of paleozoic forms. Sporangiophores of *Calamites* and *Equisetum* represent further specialization of the sporophyll of the Asterocalamitaceae.

PTEROPSIDA

The pteropsid organization is the most widespread in the vegetable kingdom. Having originated, like the other types of higher plants, during the Devonian period, the Pteropsida proved both persistent and plastic. This may be explained mainly by the peculiarities of their megaphylls which, distinguished from the phylloids of Lycopsida and meiophylls of Tmesopsida and Sphenopsida, proved capable of very diverse structural and functional modifications. This resulted in a great wealth of forms.

FILICINAE. Similar to the other types of higher plants, the Pteropsida originated from psilophytes. Members of the sub-class Primofilices were still closely related to psilophytes and occupied, in many respects, an intermediate position between the latter and true ferns. The only heterosporous representative of primofilices

known is the Devonian genus *Archaeopteris,* segregated into the order Archaeopteridales. *Archaeopteris* was probably the ancestor of primitive Pteridospermae. In the opinion of Arnold (1947), on a morphological basis *Archaeopteris* could be classified between homosporous ferns and the Pteridosperminae or even between Pteridosperminae and Psilophytales. But because the leaves of *Archaeopteris* are decidedly fern-like and differ greatly from branches of psilophytes, this genus (*Archaeopteris*) can not be considered an intermediate form between the Psilophytaceae and seed plants. It is more probable that *Archaeopteris* represented a transitional form between the ancient ferns of the order Zygopteridales and the primitive pteridosperms. *Archaeopteris* had not yet reached the ovule stage, however, and in this respect there exists a considerable gap between *Archaeopteris* and pteridosperms.

The order Ophioglossales, segregated by us into an independent sub-class Ophioglossinae, consists of direct descendants of primitive paleozoic ferns. Ophioglossinae are extremely archaic in the structure of their gametophyte, the development of their sporangia and certain other features. Nevertheless and in spite of some primitive characteristics, there are also signs of high advancement, and in this regard the Ophioglossinae represent an excellent example of rather prominent evolutionary heterochrony of various parts of the plant—" the law of corresponding stages in evolution ".

Of particular interest is the sub-class Noeggerathiinae. A series of exceedingly interesting fossil plants was included in this class, described from the Carboniferous and Triassic periods in Europe and Asia. Noeggerathiinae have been classified as an independent order, Noeggerathiales, the systematic position of which remained for a long time obscure. Noeggerathiinae were most frequently associated with the Sphenophyllales. The systematic position of the Noeggerathiinae has been conclusively clarified by Hirmer (1941) who proved that they represent a separate group of eusporangiate ferns. The Noeggerathiinae are heterosporous ferns with pinnate leaves. While they display obvious signs of affinity with the Marattiinae, they are more closely related to the Archaeopteridales. By the shape of their leaves, position of their fertile pinnules and sporangia, heterospory and shape of their megaspores, they are fairly closely related to the genus *Archaeopteris* (Hirmer, 1941).

Another relatively primitive eusporangiate group of ferns is the

sub-class Marattiinae, in which may be included only a single order, Marattiales. As a rule, the orders Marattiales and Ophioglossales are combined into a single sub-class, Eusporangiatae, though such combination of these two orders is artificial. The order Marattiales differs greatly from the Ophioglossales by the abaxial position of its sporangia, by the peculiar structure of the stem and morphology of the leaves. Each of these two orders represents an independent line in the evolution of eusporangiate ferns, and originated, undoubtedly, from separate representatives of the primeval ferns.

The most advanced sub-class among the ferns appears to be the Leptofilices (orders Filicales, Marsileales and Salviniales). Heterosporous orders Marsileales and Salviniales are frequently combined into a common order, Hydropteridales, entirely artificially. These orders have little in common with each other and originated from different families of homosporous leptosporangiates. The Marsileales originated from homosporous ancestors of the type of the contemporary Schizaeceae (vide Campbell, 1940; Bower, 1926, 1935; Eames, 1936; Christensen, 1938). This is evidenced by the obvious resemblance in the development of the sporocarp of the Marsileales and the fertile segment of the leaf of *Schizaea*. The sporocarp of the Marsileales may be considered a more specialized fertile segment of a leaf, of the type common to Schizaeaceae. The early stages of development of sporangia of Marsileales and Schizaeaceae are also very similar. Although the annulus in the majority of Marsileales became entirely reduced, the microsporangia of *Pilularia americana* have a very pronounced oblique annulus, in structure typical of the Schizaeaceae. Definite traits of resemblance have been observed also in the anatomical structure of the vegetative organs of these two families. The juvenile leaves of *Marsilea* and the awl-shaped leaves of *Pilularia*, which resemble them and are neotenic in origin, have their own analogues in species of *Schizaea* (e.g., *S. pusilla*) adapted to humid conditions. The venation in the leaves of *Marsilea* is dichotomous, as in the majority of the Schizaeaceae. And finally the structure of the stele in the stem of *Marsilea* resembles that of certain Schizaeaceae, e.g., *Anemia hirsuta*. All this is evidence of probable, and not very remote, phylogenetic connection between Marsileaceae and Schizaeaceae. The order Salviniales, however, originated in all likelihood from

the Hymenophyllaceae (vide Lotsy, 1909; Bower, 1926; Rao, 1935; Eames, 1936; Campbell, 1940; and others). In the Hymenophyllaceae cup-shaped, cylindrical, campanulate or bilabiate indusia are typical. In the Salviniales, however, in the course of complete adaptation to an aquatic habit, the indusia became entirely closed apically, forming a globose or ovoid capsule which offers complete shelter to developing sporangia. Such enclosed sori are frequently called " sporocarps ", although the sporocarps of the Marsileaceae represent a fertile segment of a complex leaf, not individual sori as they do in the Salviniaceae. Ontogenesis of a sorus in Salviniaceae clearly indicates that the closed indusium in the Salviniaceae originated from the cup-shaped or campanulate indusium of their ancestors, the Hymenophyllaceae. An indusium developing in Salviniaceae initially is a small ring at the base of the sorus, which by terminal growth becomes first cup-shaped, then tapered apically and finally closed, thus covering and completely surrounding the sorus. The relationship between the aquatic order Salviniales and the hygrophilous family Hymenophyllaceae may be confirmed also by the presence of graduate sori with an elongated receptacle, by the position of sori on leaves, by the vestigial oblique and complete annulus on microsporangia of some species of *Azolla,* by characteristic venation of the leaves, by the structure of leaf traces, etc. Thus the two heterosporous families Marsileaceae and Salviniaceae are of distinct origin, and their combination into one order is inadmissible in the phylogenetic system. One of the serious shortcomings in Zimmermann's system (1930, 1938) is the combination of these two families in the group Hydropterides which he derives directly from the Archaeopteridales. At the same time combination of these two families with the homosporous Filicales, as has been suggested, for instance, by Eames (1936) and Smith (1938), is not expedient. Christensen (1938) segregated Salviniaceae and Azollaceae into an independent order Salviniales, although he classified Marsileaceae among the Filicales next to the family Schizaeaceae. Subsequently, however, the order Marsileales was segregated by Dickson (1946).

GYMNOSPERMAE. The Gymnospermae originated from some ancient and primitive group of heterosporous ferns, most probably from the Archaeopteridales. The origin of seed plants is allied with the origin of an ovule; the latter is the principal feature which

distinguishes ferns from seed plants. The origin of the ovule was closely connected with the evolution of heterospory in Pteropsida and represents a definite aromorphosis which has enabled seed plants to emerge supreme in the struggle for existence over all the other telomous plants. An ovule is a specific type of megasynangium, in which there is a division of functions between the central fertile megasporangium (nucellus) and the surrounding ring of sterile sporangia which forms a protective integument. Primitive ovules of pteridosperms frequently retain rather apparent traces of their synangial origin (multi-chambered integuments and free ends of sterile sporangia). The primitive seed plants did not yet have a true seed with the dormant embryo quiescently reposing in it.

The class Gymnospermae is usually subdivided in different ways by various authors. It was Nathorst who proposed division of the Gymnospermae into two groups, Cycadophyta (Pteridospermae, Bennettitales and Cycadales) and Coniferophyta (remaining orders). Jeffrey (1917) named these two groups Archigymnospermae and Metagymnospermae, while Sahni (1920) suggested two more suitable names, Phyllospermae and Stachyospermae. Others have subdivided gymnospermous plants into three or four groups, whereby some of them eliminated the general name, Gymnospermae. Some authors, e.g., Berry (1917), segregated the independent group Pteridospermophyta, equivalent to Cycadophyta and Coniferophyta, while others, e.g., Gaussen (1944), segregated, on the contrary, Gnetophyta, leaving Cycadophyta in its former capacity. According to Pulle (1937), however, all seed plants ought to be subdivided into Pteridospermae, Gymnospermae (in the narrower sense of the word), Chlamydospermae (*Ephedra, Gnetum* (Gnetaceae) and *Welwitschia*) and Angiospermae. Consequently neither Pulle nor Berry had a common name for the entire branch of development of gymnospermous plants, beginning with Pteridospermae and terminating with Chlamydospermae. The wisdom of abolishing an overall group, Gymnospermae, is definitely questionable, since such a move disturbs the phylogenetic structure of the whole subdivision Pteropsida if the latter is divided, to reflect the three basic stages of its evolution, into classes Filicineae, Gymnospermae, and Angiospermae. We propose, therefore, to examine Pteridospermae and Chlamydospermae along with Phyllospermae and Stachyospermae as subclasses subordinate to

the class Gymnospermae. We consider the classification by Arnold (1942) unsuitable in this respect. He subdivides Pteropsida into five classes: Pteridophyta, Cycadophyta, Coniferophyta, Chlamydospermophyta and (with a question mark) Angiospermophyta. Thus Arnold's system eliminates Gymnospermae as an overall class, and this can hardly be considered a step forward. Still less conclusive if the system of Lam (1948), in which all the higher plants are divided into Eucormophyta, Palaeocormophyta, Mesocormophyta and Neocormophyta. Lam divided the Palaeocormophyta into Lycopsida, Sphenopsida and Pteropsida (in a limited sense). Within his Pteropsida are included the Filices, Hydropteridales, Noeggerathiales, Caytoniales and Pteridospermales. Lam further divided the Mesocormophyta into Cycadopsida and Coniferopsida; and the Neacormophyta into Protangiospermae and Angiospermae, including in the former the Chlamydospermales (Gnetales), Sarcopodales (?) [5] and Verticillatae (*Casuarina*). According to Lam, the genus *Exocarpus* probably represents a transitional form between "protoangiospermous gymnosperms" and the "Monochlamydeae". In spite of modernized nomenclature and constant reference to "new morphology", Lam's system revived errors of the prephylogenetic period in the historical development of systematics of higher plants. Suffice it to say that Lam allies *Casuarina* with *Ephedra* and looks for transitional forms from his own Protangiospermae to "Monochlamydeae". In all, Lam's system is too formal and artificial.

In my opinion, subdivision of class Gymnospermae into the subclasses Pteridosperminae, Phyllosperminae, Stachyosperminae and Chlamydosperminae is most constructive and useful for phylogenetic systematics.

Pteridosperminae. The Cycadofilicales, including the Caytoniaceae, is placed in the sub-class Pteridosperminae. The order Cycadofilicales is undoubtedly the most primitive among the gymnospermous plants. The most primitive family in this order appears to be the Lyginopteridaceae. The genera *Heterangium* and *Tetrastichia* (Gordon, 1938) of this family and the genus *Sutcliffia* of Medullosaceae appear to be the only authentically known representatives of seed plants in which we find a protostelic stem in an

[5] *Sarcopus* = *Exocarpus* ex Santalaceae.

adult plant. In many respects the structure of the stem in *Heterangium* suggests primitive protostelic ferns or even psilophytes, except that the capacity of developing secondary tissues had already been attained. In all the rest of Lyginopteridaceae a mesarch siphonostele with a comparatively large pith is observed, as well as a relatively strongly developed secondary wood and a thick cortex. The structure and position of ovules in Lyginopteridaceae is of particular importance for the phylogeny of Pteridosperminae and other Gymnospermae. The most primitive position of ovules is marginal on the leaf. The other types of ovule distribution developed from this marginal type. In the evolution of ovule distribution in Lyginopteridaceae two tendencies are known: *a*) from the marginal to the laminal, and *b*) from the marginal to the secondary-terminal. Phylogenetically the secondary terminal position of ovules is very important because it brought about the appearance of the Stachyosperminae. In *Aneimites fertilis,* for example, the ovules were attached to apices of narrow pedicels which correspond to reduced and modified leaflets; i.e., fertile leaflets of a pinnate leaf became simplified and transformed into pedicels, similar to those in Ginkgoales and Cordaitales. The formation of a special cup-shaped covering of an ovule, the cupule, in a score of Lyginopteridaceae, e.g., *Calymmatotheca,* is also very important from the phylogenetic viewpoint.

H. R. de Haan (1920) advanced the idea in favour of the origin of the cupule in Pteridospermae from modified pinnules of a compound sporophyll. Andrews (1940), however, proposed that the cupule originated from those sterile telomes which, in his opinion, surrounded the fertile telomes of the future ovules. In his opinion, it is quite in order to assume that the sporangia-bearing organs in the ancestors of Pteridospermae consisted of a terminal group of sporangia surrounded by an annulus of sterile telomes; thence the sporangia united into a synangium and thus gave birth to an ovule with a single integument, while sterile telomes flattened out and, having coalesced with one another, formed the segments of the cupule. The telome origin of the cupule could be considered as probable only if we were successful in proving that the cupule in the Pteridosperminae developed earlier than did the leaves. The cupule evolved, however, in that stage of evolution when the flat dorsiventral leaves had already definitely developed. In addition,

cupules are found in only a few pteridosperms, and ovules, devoid of cupules, do not show any rudiments. Thus the conclusion may be drawn that the cupule in pteridosperms may be a new organ, and not a heritage of psilophytaceous ancestors. The structure of the cupule itself indicates that it has developed from part of the leaf and not directly from telomes. Andrews himself referred to the large size of the lobes in the cupule of *Magatheca Tomasii*. These lobes are, however, of foliar instead of telomous nature. The capitate glands of the cupule in *Calymmatotheca* and its anatomical structure confirm the foliar nature of a cupule. And finally the existence of cupules with a few ovules instead of one is conclusive evidence of their origin from modified segments of compound leaves. Ovules of the genus *Gnetopsis* known from the Upper Carboniferous, three or four of them attached to each cupule, present an interesting example of this. A still more interesting example is *Calathospermum scoticum*, found in the Lower Carboniferous of Scotland (Walton, 1940, 1949). The cupule in this case is very large, up to 4.5 cm. in length, and divided almost to the base into six segments. Each cupule carries five or six conducting bundles distally which are united at the base of the cupule. The pedicel of a cupule has a conducting bundle which is crescentric in cross section and hence is evidently a part of the rachis of a leaf. In each cupule there were several dozen ovules. It is worthy of note that the pedicels of the upper ovules were attached to the margins of the segments of the cupule, and consequently their marginal position is still preserved. The marginal position of ovules still observed in *Calathospermum* is the best demonstration of the fact that segments of a cupule represent modified segments of a leaf. In Walton's opinion (1949), *Calathospermum* may be considered the ancestral seed-bearing type, from which other types developed. Moreover, this may be regarded as the more probable, in view of Andrew's opinion (1948) that *Calathospermum* appears to be the cupule of *Tetrastichia bupatides*. If the number of ovules is reduced to three or four, then the type *Gnetopsis* develops; should a single ovule have remained, however, we obtain the *Lagenostoma* type (ovule of genus *Calymmatotheca*). In this way a "cupule" represents the outer covering of an ovule which then is, in contrast to the integument or inner nucellar covering, of foliar and not sporangial origin. The cupule for the

first time originated in Lyginopteridaceae but it is not seen exclusively in these primitive gymnosperms. The future destiny of a cupule is of outstanding interest for the evolutionary morphology and phylogeny of the spermatophytes. The comparative morphology of fossil and contemporary ovules brings us to the deduction that the cupule in the process of evolution coalesced with the integument and finally became transformed into an outer integument. It was M. C. Stopes (1905) who examined the outer fleshy layer (sarcotesta) of the seed of *Cycas* and explained it as a formation homologous to the outer integument (i.e., cupule) in *Lagenostoma* . . . . This homology may be seen clearly in a score of other gymnosperms, examples of which we shall cite later. Not only did various periovular formations in gymnosperms develop from cupules, but, in our opinion, the outer integument of angiosperm seeds did so also. This hypothesis is excellently exemplified by cases of virescence of ovules in which a foliolar expansion of the outer integument is associated with a rudimentary inner integument. Gaussen (1946) also recognizes the foliolar nature of the outer integument in angiosperms. A most interesting and important peculiarity of ovules in the pteridosperms is that no embryo has ever been found in them. This conspicuous absence of an embryo may be explained by the fact that in these primitive Gymnospermae a continuous development of the zygote, typical of the ferns, is still preserved. Thereby the definite period of rest, with which the concept of a mature seed is associated, is completely eliminated. Thus the ovules in pteridosperms differ from the ovules of more advanced Gymnospermae by an early development of all tissues. Already before fertilization the ovule ceased to develop and ultimately lost its capacity to grow in size. Consequently pteridosperms as well as Cordaitales did not have real seeds, although they had ovules. They were ovular plants, though not entirely spermatophytes! That is precisely why Emberger (1945) segregated Pteridospermae, Caytoniaceae and Cordaitales into a separate group, Prephanerogames, i.e., those which preceded Phanerogames (true spermatophytes). Such a division of seed plants, however, is rather artificial, for it widely separated Corditales from the closely allied Ginkgoales and Coniferales.

In the family Medullosaceae both vegetative and reproductive organs are more advanced than in the Lyginopteridaceae. The

most primitive representative of Medullosaceae is the genus *Sutcliffia* from the Lower Carboniferous of England. *Sutcliffia* represented, probably, one of the transitional forms from the primitive Lyginopteridaceae of the type *Heterangium* to the typical Medullosaceae. The ovules, which belong, as assumed, to Medullosaceae, are combined into an artificial group, the Trigonocarpales. It is usually considered that the megasporangium (nucellus) is free from the integument in these ovules. It is further assumed that the ovules in Medullosaceae have a double conducting system which supplies not only the integument, as in Lyginopteridaceae, but also the nucellus. What is usually taken for integument of the ovule represents, however, in our opinion just a modified cupule, whereas the exterior vascularized layer of " nucellus " is in reality the true integument. The wall of a true megasporangium, and of sporangia generally, can not be vascularized because walls of sporangia never have a conducting system. The conducting system may be found only in the integument which developed as a result of coalescence of the ring of sterile sporangia. Separate bundles of the conducting system of the integument correspond to the individual bundles of sporangia. Individual bundles of these integumentary conducting traces did not enter the locules, although during the process of sterilization of the sporangium and modification of its function, the conducting trace did so ingrow. The cupule of *Trigonocarpus* is greatly modified and opens superiorly into a long canal. The latter is differentiated into three layers, of which the outer fleshy layer is provided with six longitudinal vascular bundles. There is a definite resemblance in the anatomical structure of the cupule of *Trigonocarpus* with the petiole of the leaf in *Medullosa anglica.*

In the still more advanced Upper Triassic family Peltaspermaceae (*Lepidopteris*) the ovules were suspended from the lower surface of a peltate discoidal cupule around its pedicel. In the family Corystospermaceae the cupules appear helmet-shaped, campanulate or bivalved, and contained an ovule each. According to Andrews (1948), the early Triassic Corystospermaceae represent a connecting link between the late paleozoic Pteridospermae and the Caytoniaceae. The cupules of Corystospermaceae represent a prototype of the closed cupules of Caytoniaceae, although, in distinction from the latter, they had become monospermous. Micro-

spores in Corystospermaceae and Caytoniaceae are of one type, those of both families having two lateral wings. The Caytoniaceae is closely associated with the Corystospermaceae, and there seems to be no reason whatever for segregating the former to an independent order (Caytoniales), hence it is here included in the Cycadofilicales.

Phyllosperminae. The sub-class Phyllosperminae is phylogenetically derived from pteridosperms of the Medullosaceae type. In particular the anatomical structure of the stems of the Cycadaceae strongly resembles that of the Medullosaceae. It is rather interesting that among the Cycadaceae both monocambial and polycambial forms are observed. In *Zamia, Stangeria,* and *Dioon* the original cambium retains its activity during the entire life of a plant. In other Cycadaceae, however, the activity of the original cambium lasts for a short time only, and further thickening of the stem is caused by subsequent concentric cambial ring development in the cortex. Monocambial stems of the *Zamia* type are more primitive than polycambial. In Cycadaceae as well as in angiosperms, polycambial stems developed during evolution from the monocambial. It is of interest that while the secondary woody tissue in polycambial cycads consists entirely of tracheids with bordered pits, that in the monocambial genera *Zamia* and *Stangeria* contains more primitive scalariform tracheids with thickenings similar to those of metaxylem. The ovules in the Cycadaceae are of the same type as those in Medullosaceae, though more elaborate. Systems of conducting bundles are contained in both the outer and inner fleshy layers of the ovule's integument. The outer fleshy layer represent the greatly modified cupule which has, in contrast to that of the Medullosaceae, fused with the integument. The cupular nature of this layer is evidenced not only by the presence of an independent conducting system, but also by the fact that mucilage ducts run through this layer.

Notwithstanding the indubitable affinity between Bennettitaceae and Cycadaceae, their relationship is only through sharing a common origin in some group of Pteridosperminae resembling the more primitive Medullosaceae. The divergence between these two groups probably occurred at a very early stage, as indicated by different types of stomata, haplocheilous in Cycadaceae and syndetocheilous in Bennettitaceae (Florin, 1933), and also by differ-

ent types of strobili. It may be assumed that strobili in Cycadaceae and Bennettitaceae arose independently. Besides, in distinction to Cycadaceae, the cupules in Bennettitaceae are free, not fused with the integument. This condition may be explained probably by the ovules being sheltered with interseminal scales which are expanded distally to provide a protective covering.

Stachyosperminae. Compared with Phyllosperminae, sub-class Stachyosperminae represents a different line of morphological evolution. In the Phyllosperminae the leaves were for the most part pinnate, and the stems slightly branched or even unbranched, whereas in Stachyosperminae the foliar organs were simple and usually entire, and the stems ramose. If in the one case a large photosynthesizing surface had been attained by formation of large dissected leaves, then in the other the photosynthesizing surface was acquired as a result of branching of the stem. The same physiological effect may thus have been attained by two distinct methods of morphological differentiation. The second method appears to be the more fruitful of evolutionary possibilities. The evolution of strobili in Stachyosperminae also took a different course.

The most primitive family of the order Cordaitales is the Pityaceae, known from the Upper Devonian and Lower Carboniferous. Arnold associates the family Pityaceae phylogenetically with certain genera of the family Calamopityaceae of the Pteridospermae; it probably originated from species closely allied with the latter family, though its members were more advanced. The relationship of the Pityaceae, just as that of the closely allied Poroxylaceae, with their pteridosperm predecessors can be established at present only from a study of the stru:ture of vegetative organs, since the ovules of these plants are unknown. The ovules in the family Cordaitaceae are constructed, however, according to the same general plan as those of the Pteridosperminae and are provided with free cupules. The Cordaitaceae are typically stachyosperminous in the structure of their strobili, with terminal position of their ovules and microsporangia. Florin (1939) considers the position of the ovules of the Cordaitaceae primarily terminal and derives it directly from the terminal position of sporangia of the Psilophytales. The Cordaitaceae, however, may be associated with the Psilophytaceae only through two intermediate groups—Primofilices

and Cycadofilicales. The position of sporangia and ovules in Cordaitaceae appears to be secondarily terminal and may be derived from their similar position in certain of the pteridosperms.

The order Ginkgoales is undoubtedly closely allied with the Cordaitales. The position of microsporangia and ovules in both groups is secondarily terminal and developed from the secondarily terminal position in a certain group of pteridosperms. Both groups developed from a common trunk, going back to the pteridosperms. The double leaf-trace and other conclusive morphological characters closely associate Ginkgoales and Cordaitales with the seed ferns. The Cordaitales and Ginkgoales originated, however, from Pteridosperminae in which microsporangia were still free and had not united into synangia. In the Ginkgoales, in distinction to the Cordaitales, the cupule was preserved in the shape of a collar at the base of the ovule. The cupular nature of the collar may be confirmed by the occasional occurrence of teratological forms intermediate between a normal collar and more or less modified foliage leaves bearing ovules. The transition of the collar into a green leaf confirms its foliar, namely cupular, nature.

The Coniferae are descendants of the Cordaitales. Some contemporary conifers strongly resemble the latter in habit; the leaves of *Agathis australis,* for example, are almost indistinguishable from those of Cordaitales. In addition, the Araucariaceae have retained the multiseriate crowded and contiguous bordered pits typical of Cordaitean wood. The phylogenetic link is also inferred from a study of reproductive organs. The structure and position of strobili may, however, be greatly obscured in the conifers because of remote processes of reduction and specialization that make comparisons difficult. Spike-like inflorescences (compound strobili or metastrobili) of *Podocarpus spicatus* and *Podocarpus andinus* have retained their original almost cordaitaceous character. The small microstrobili of these two species, similar to those of the Cordaitales, are sessile in the axils of bracts along the main axis. Among the remaining species of the genus *Podocarpus* there may be observed a gradual reduction in number of microstrobili on the main axis until ultimately there remains but one. At the base of such a microstrobilus there are preserved, however, the sterile bracts of vanished microstrobili. Primitive inflorescences (metastrobili) occur also in *Sciadopitys, Austrotaxus, Cephalotaxus* and *Amento-*

taxus, but here they are of a rather more modified type than in the species of *Podocarpus.* In the remaining conifers, as a result of reduction of the original cordaitaceous inflorescence, only isolated microstrobili (i.e., inflorescence with a single microstrobilus) remain. The evolution of microsporophylls in the Coniferae is also rather interesting. Dorsiventral microsporophylls developed in the process of evolution from the original radially symmetrical microsporophyll of the Cordaitales and Ginkgoales type. From the very primitive microsporophyll of the *Cephalotaxus* type with pendant microsporangia, as in the Ginkgoales, there originated the peltate microsporophylls of *Nothotaxus* and *Taxus,* and from the latter, as a result of reduction of adaxial sporangia, developed the dorsiventral type (Takhtajan, 1941, 1943; Florin, 1948). In the dorsiventral type of microsporophyll, the sterile portion of the apex frequently elongates and expands into a blade, and in this manner a secondary laminal position of microsporangia is initiated, seen particularly in the Taxodiaceae and Cupressaceae. Thus in conifers a secondary laminal position of microsporangia developed from a secondary terminal one.

The evolution of megastrobili is rather important in the phylogeny of the conifers. The most primitive type of female inflorescence is observed in both the above-mentioned species of the genus *Podocarpus.* On the main axis of the cordaitaceous spike-like inflorescence are attached greatly reduced, though still free, megastrobili. In the remaining species of *Podocarpus,* a process of reduction in the number of megastrobili on the main axis is observed (Wilde, 1944). As a result of this process of reduction, megastrobili of the *Taxus* type developed, resulting in a single erect ovule. In the family Podocarpaceae, however, another trend of evolution may also be observed. It is not expressed in a reduction of the number of separate megastrobili, but in the shortening of the internodes of the main axis, resulting in the formation of a compact cluster of megastrobili or a "cone". In the genus *Saxegothaea* the upper megastrobili became contiguous to one another and thus formed a small cone. These two trends in the evolution of female inflorescences became prominent also in the related families Taxaceae and Cephalotaxaceae. In *Nothotaxus* and *Taxus,* owing to reduction, solitary monospermous megastrobili formed, while in *Amentotaxus* and *Cephalotaxus* small cones developed. In all the

remaining conifers, however, evolution ran in a trend to increased compactness of the entire megasporangiate branch, while diminution in the number of parts was of secondary importance. As a result, various kinds of cones formed, which consisted of protective scales (bracts) with greatly modified and reduced megastrobili (the so-called ovuliferous scales) in their axils. The question of the nature of the ovuliferous scale in conifers has long been one of the disputed problems in botanical morphology. As a result of investigations, begun in the forties of the last century (works of Alexander Braun), it was finally proved that the "ovuliferous scale" appears to be a dwarf shoot (brachyblast) representing a reduced megastrobilus. In a paleozoic conifer (*Lebachia*) there emerged from the axils of the bifurcated bracts a true small megastrobilus, on which scale-like megasporophylls and sterile leaves were situated spirally, just as in the Cordaitales, while in the genus *Ernestiodendron* a great many megasporophylls were found on the axis of the brachyblast. Ultimately, out of such a small megastrobilus, owing to the diminished number and size of megasporophylls, and due to the coalescence of the upper megasporophylls, there formed a flat ovuliferous scale with a secondary-laminal position of ovules. In the process of further evolution the ovuliferous scale fused with the bracts in many conifers.

The most primitive group known among the conifers is the family Voltziaceae which had characteristic primitive megastrobili that frequently were still almost cordaitaceous. The Voltziaceae can not truly be considered the common ancestor of all more recent conifers. Their acicular leaves were considerably more reduced than those of the contemporary genera *Agathis* and *Podocarpus*. This bears witness to the fact that the "coniferous needle" (acicular leaf), typical of most contemporary Coniferales, originated in the very beginning of evolution of this order. Although the Voltziaceae still frequently retained primitive megastrobili, they already possessed a "cone" and in this feature were actually more specialized than *Podocarpus spicatus* and *Podocarpus andinus*. Which, then, are the most primitive species among contemporary conifers, and in which directions has their evolution proceeded? Formulating a system of conifers depends upon appropriate answers to these questions. At present there is unanimity of thought neither about the trends in evolution of the conifers, nor about their

system. In accord with many contemporary authors, Araucariaceae and then Podocarpaceae are here considered the most primitive families among modern conifers. This position of these families is recognized in the systems of Yarmolenko (1933) and Pulle (1937). The primitiveness of the Araucariaceae may be inferred by the cordaitean type of leaves in *Agathis* and some species of *Araucaria,* by the multiseriate bordered pits in the tracheids, by the presence of many vegetative (prothallial) cells in the male gametophyte, by the distribution of archegonia, et simile. The Araucariaceae probably originated from an unknown and closely related ancestor of the Voltziaceae which had more primitive leaves than the latter. The Mesozoic genus *Schizolepis* is probably an intermediate type; Florin (1944) considers the ovuliferous scale of this genus the prototype of the ovuliferous scale of the Araucariaceae. In *Schizolepis* the ovuliferous scale is still free and consists of three megasporophylls, each bearing a single apical ovule. In the course of evolution each megasporophyll fused with the distal sterile scale of the megastrobilus. Hence ovule distribution became reduced to a solitary one. In *Araucaria bidwillii* the conducting traces of sterile and fertile scales have still completely preserved their individuality. Investigation of the conducting system has shown, moreover, that in the past the fertile scale bore three ovules, as in *Schizolepis* (vide Wilde and Eames, 1948).

Closely related to the family Araucariaceae and undoubtedly of common origin with it is the family Podocarpaceae. Although the bordered pits of the tracheids in Podocarpaceae are usually distributed uniseriately, some species of *Dacrydium* have sections on the tracheids where bordered pits are crowded similarly to those in the Araucariaceae and Cordaitales. The genus *Saxegothaea* is closely allied with *Araucaria bidwillii* in structure of the wood. Compact cones are present also in the genus *Podocarpus.* In some species the female inflorescence is reduced to a single ovule, as in *Taxus.* The male gametophyte in *Podocarpus* is primitive and produces a considerable number of vegetative cells.

The family Cephalotaxaceae (*Cephalotaxus*) is closely related to the family Podocarpaceae, especially to section Stachycarpus of genus *Podocarpus,* and in some respects occupies an intermediate position between primitive Podocarpaceae and Taxaceae. Pulle (1937) combines Cephalotaxaceae and Taxaceae into the order

Taxales, while in the system of Buchholz (1948) they are classified side by side. Microsporophylls in the Cephalotaxaceae are of primitive construction, bearing two to five pendant microsporangia. The cones of the Cephalotaxaceae have greatly reduced megastrobili.

The family Taxaceae originated from the Podocarpaceae. The ancestors of the Taxaceae must have had wingless microspores. Among contemporary Podocarpaceae only a single genus (*Saxegothaea*) is devoid of bladders on the microspores. *Austrotaxus* and *Amentotaxus* still preserve the spike-like male inflorescence, while in *Taxus, Nothotaxus* and *Torreya* the microstrobili are already solitary. Megastrobili in the Taxaceae are greatly reduced, devoid of the fertile scale, and in the genus *Taxus* became reduced so far as to leave only a solitary ovule. In a work dedicated particularly to the phylogeny of the Taxaceae, Florin (1948) asserts that the Taxaceae are not all allied with the other contemporary families of conifers but are of an entirely independent origin. He was motivated in his assertion by the fact that female cones are missing from the Taxaceae and are substituted by solitary and apical ovules. Florin disagrees with Pulle's theory in which the Taxaceae are regarded as having the most reduced type of female cone. He thereby supports the theory of Sahni (1920) who assumed that *Taxus, Torreya* and *Cephalotaxus* differ structurally to such an extent from the remaining conifers that the aforenamed genera should be segregated into an independent group (Taxales) equivalent in systematic rank with the Coniferae. Florin's assertions must, however, be entirely disregarded. The evolution of the megasporangiate branches of the Podocarpaceae indicates the course which brought about the formation of reduced cones in Taxaceae. Besides, the cones of the genus *Torreya* consist of two ovules in the axils of small leaves. As far as *Taxus* is concerned, the ovule is only pseudo-terminal. In reality the solitary ovule of *Taxus* is in the axil of one of the uppermost scaly leaves of the main shoot; in the process of development it displaces the blind apex of the shoot to the side and thus occupies a pseudo-terminal position. The Taxaceae are thus descendants of the Podocarpaceae, which fact is confirmed by the structure of the wood (Yarmolenko, 1933).

The large family Pinaceae probably originated directly from the

Voltziaceae. The Pinaceae is not an ancestor of the Araucariaceae, as was assumed by Jeffrey (1917) and others, e.g., Buchholz (1948) who in the most recent system of conifers distributes the families as follows: Pinaceae, Araucariaceae, Taxodiaceae, Cupressaceae, Podocarpaceae, Cephalotaxaceae, Taxaceae. In my opinion, neither did the family Pinaceae originate from *Araucarioxylon*, as proposed by Yarmolenko (1933). The families Araucariaceae and Pinaceae are instead of independent origin from different groups of fossil conifers. Notwithstanding their free and more primitive ovuliferous scale than that of the Araucariaceae, the Pinaceae appear to have originated from more advanced species with acicular leaves and compact cones.

Arnoldi (1900) early expounded the necessity of segregating the monotypic and rather unique Japanese genus *Sciadopitys* into an independent family. The genus *Sciadopitys* differs from the remaining representatives of the family Taxodiaceae, not only by important external morphological characteristics but also by the structure of its wood, by the distribution of its archegonia and by the development of the embryo. In the systems of Pilger (1926) and Buchholz (1948) the genus *Sciadopitys* is segregated in a separate subfamily (Sciadopityoideae), but Hayata (1931), Yarmolenko (1933) and Domin (1938) segregate it in a separate family (Sciadopityaceae). Yarmolenko derives this family from *Dadoxylon* via *Xenoxylon* and *Walchioxylon*, and thereby suggests its origin independently of other conifers. The common origin of the Sciadopityaceae and Taxodiaceae from ancient Pinaceae may, however, be considered more probable. Microsporophylls in *Sciadopitys* are of the same structure as in the Pinaceae, though microspores are devoid of bladders.

According to Yarmolenko (1933), the family Taxodiaceae originated from Pinaceae with accompanying loss of resin canals. The presence of a representative of the Taxodiaceae in the Jurassic (*Elatides*) bears witness to the very early divergence of the two families. Although in contrast to the Pinaceae the ovuliferous scales in Taxodiaceae have entirely fused with the bracts, their microspores are devoid of bladders. Taxodiaceae originated probably from the earliest and most primitive Pinaceae which at that time did not possess any bladders on the microspores. The genus *Athrotaxis*, endemic to Tasmania, is noteworthy because it, by

some rather important characteristics, represents an apparent transition towards the family Cupressaceae. In view of the distribution of archegonia and numerous other characteristics, the family Cupressaceae is very closely related to Taxodiaceae and has, no doubt, originated from the latter. It is also assumed that the Sequoideae lie in the chain of ancestry of the Cupressaceae.

Chlamydosperminae. The most advanced group of gymnosperms appears to be the sub-class Chlamydosperminae. The genera *Ephedra, Welwitschia* and *Gnetum* have been most frequently combined into one group (Gnetales). Schaffner (1929), however, and subsequently Arnold (1948), have subdivided this group into two orders, Ephedrales (Ephedraceae) and Gnetales. Pulle (1937) also subdivides his Chlamydospermae into two orders, although with a different classification of families, including the Ephedraceae and Gnetaceae in the Gnetales, but segregating the Welwitschiaceae to an independent order (Welwitschiales). In our opinion, however, the three aforementioned families differ so greatly from one another that it is more plausible to regard them as entirely separate orders.

The absence of paleobotanical data makes it difficult reliably to clarify the origin of the Chlamydosperminae, as has been attained, for example, in the phylogeny of the conifers. In analyzing the strobili of the Chlamydosperminae, however, we invariably arrive at the conclusion that their ancestors had bisexual (amphisporangiate) strobili. Of all fossil and even contemporary gymnosperms, only the Bennettitales are known to have had bisexual strobili. It would thus appear conclusive that Chlamydosperminae either originated and developed directly from Bennettitales or were of a common origin, both groups coming from some more primitive group with bisexual strobili. Chlamydosperminae have a great deal in common with the Bennettitales in spite of their vast differences in habit.

The dichasial branching of an inflorescence (metastrobilus), unknown in any other contemporary gymnosperms, occurred in *Wielandiella augustifolia,* as pointed out by Pearson (1929). This resemblance extends further, since micropylar tubes, typical of the Chlamydosperminae, were found also in the Bennettitales as well as in certain Pteridosperminae. The analogy between the ovules

of *Gnetum* and those of some of the Bennettitales extends even to details (Berridge, 1911; Pearson, 1929; and others). The male strobilus of the genus *Welwitschia,* with its central rudimentary ovule and the ring of microsporophylls fused at their bases, may be derived from the considerably more primitive bisexual strobili of the Bennettitales (Pearson, 1906, 1909; Arber and Parkin, 1908, 1921; Berridge, 1911; Wieland, 1916; and others). Pearson (1909), for example, considers that the "flower" of *Welwitschia* represents a greatly reduced strobilus of the Bennettitales. Zimmermann (1930) derives (with an interrogation mark) the Gnetales directly from the Bennettitales. If one were to imagine that the number of megasporophylls in a bisexual strobilus of the Bennettitales became reduced to a single one and that the basily fused microsporophylls also became greatly reduced, we would then receive a strobilus of the *Welwitschia* type. In genera *Gnetum* and *Ephedra* the number of microsporophylls in a male strobilus became reduced to one. These points all indicate that the Chlamydosperminae represent greatly reduced descendants of the Bennettitales.

ANGIOSPERMAE. From the ancient Phyllosperminae there originated not only the reduced Chlamydosperminae with their greatly simplified strobili, but also the progressive group Angiospermae. But if the Chlamydosperminae originated directly from Bennettitales, then the Angiospermae are of a common origin only. The Phyllosperminae and the Angiospermae represent a single common branch of development, the origin of which may be traced to mesozoic Pteridosperminae. The transition from Pteridosperminae without strobili towards strobilous Gymnosperminae was a tremendous evolutionary upgrade (aromorphosis) in the history of seed plants. This is one of the most illustrative examples of discontinuity in the evolution of major taxa. Species bearing transitional forms from the long sporophyll-bearing shoots of the Pteridosperminae never have, and probably never will exist. A strobilus represents a metamorphosed bud of a sporophylliferous shoot developed from it phylembryogenetically as a result of a sudden change. The flower of the Angiospermae originated from the most primitive strobili of their closest ancestors, the Bennettitales. The transition of the primitive strobilus into a flower was probably a

phylembryogenetic sudden metamorphosis from the early (embryonic) stage of development [6], analogous to the metamorphosis of the sporangia-bearing auxiblast into a sporangia-bearing branchiblast-strobilus.

Angiospermae, it seems to me, developed from the base of the Phyllosperminae stock. That was an evolutionary upgrade of still greater significance than the transition from Pteridosperminae towards the strobilous gymnosperms. Angiospermae, owing to the extreme plasticity of their vegetative and reproductive organs, became true conquerors of the land. The genesis of Angiospermae was the ultimate link in the regular chain of evolution of the vegetable kingdom of our planet.

APPENDIX I

Conspectus Systematis Telomophytarum

Division Telomophyta Takht. nomen novum (= Cormophyta Ung.)

1. Phylum PSILOPSIDA Eames (1936) p.p. (excl. Psilotales)
   - A. Class Psilophytinae
     1. Order Psilophytales—Rhyniaceae, Horneophytaceae, Yarraviaceae, Pseudosporochnaceae, Psilophytaceae, Sciadophytaceae, Zosterophyllaceae, Asteroxylaceae
2. Phylum BRYOPSIDA Takht. (1943) [7]
   - A. Class Hepaticae
     1. Order Calobryales—Calobryaceae (Haplomitriaceae)

[6] The strobilous theory of a flower, developed in the works of most advanced morphologists, Darwinists, represents a solid property of materialistic science. A flower is a specific type of a strobilus (fertile brachyblast). The axis of a flower (or of any strobilus) represents a reduced stem of limited growth, whereas stamens and carpels are modified microsporophylls and megasporophylls. This basically simple theory called forth sharp objections among many contemporary foreign morphologists (Formalists). Popular contemporary theories of the flower denying its origin from the sporangia-bearing brachyblast (strobilus) serve as one of the most obvious examples of formalism in science. B. M. Koso-Polianskii (1936) gave a splendid example of criticism of the recent formalistic "theories" on the flower, in which he defended, brilliantly, the position of dialectical materialism. The newest theoretical constructions by morphologists—formalists—"bring about a completely obscured systematics, a taxonomic chaos, because they are impotent to serve as a lever for the structure of entire systems" (Kozo-Polianskii, 1937; see also Eames, 1951).

[7] The name BRYOPSIDA was proposed in 1943 by the author (Takhtajan, 1943). Obviously independently, the same was proposed five years later by the Dutch botanist Lam (1948).

2. Order Jungermanniales
   2a. Suborder Jungermannineae—Ptilidiaceae, Lepidoziaceae, Calypogeiaceae, Cephaloziaceae, Cephaloziellaceae, Harpanthaceae, Jungermanniaceae, Marsupellaceae, Plagiochilaceae, Scapaniaceae, Schistochilaceae, Porellaceae, Goebeliellaceae, Radulaceae, Pleuroziaceae, Frullaniaceae, Lejeuneaceae
   2b. Suborder Metzgerineae—Treubiaceae, Fossombroniaceae, Pelliaceae, Blasiaceae, Pallaviciniaceae, Metzgeriaceae, Riccardiaceae
3. Order Marchantiales—Monocleaceae, Marchantiaceae, Rebouliaceae, Sauteriaceae, Targioniaceae, Corsiniaceae, Ricciaceae
4. Order Sphaerocarpales—Sphaerocarpaceae, Riellaceae

B. Class Anthocerotae
1. Order Anthocerotales—Anthocerotaceae

C. Class Musci
1. Order Sphagnales—Sphagnaceae
2. Order Andreaeales—Andreaeaceae
3. Order Bryales
   3a. Suborder Eubryineae—Fissidentaceae, Dicranaceae, Pottiaceae, Grimmiaceae, Funariaceae, Schistotegaceae, Bryaceae, Mniaceae, Hookeriaceae, Hypnaceae, etc.
   3b. Suborder Buxbaumineae—Buxbaumiaceae, Diphysciaceae
   3c. Suborder Polytrichineae—Polytrichiaceae, Dawsoniaceae

3. Phylum LYCOPSIDA Jeffrey (1899) p.p. (excl. Psilotales, Sphenophyllales et Equisetales)

A. Class Lycopodiinae
1. Order Baragwanathiales Takht. (1943)—Baragwanathiaceae (Drepanophycaceae)
2. Order Lycopodiales—Protolepidodendraceae, Lycopodiaceae
3. Order Selaginellales—Selaginellaceae, Miadesmiaceae
4. Order Lepidodendrales—Lepidodendraceae, Sigillariaceae, Bothrodendraceae, Lepidocarpaceae

5. Order Isoetales—Pleuromeiaceae, Isoetaceae

4. Phylum TMESOPSIDA Takht. (1941)
   A. Class Psilotinae
      1. Order Psilotales—Psilotaceae

5. Phylum SPHENOPSIDA Scott (1909) p.p. (excl. Psilotales)
   A. Class Equisetinae
      1. Order Hyeniales—Hyeniaceae
      2. Order Pseudoborniales—Pseudoborniaceae
      3. Order Sphenophyllales—Sphenophyllaceae (incl. Tristachyaceae et Cheirostrobaceae)
      4. Order Equisetales—Asterocalamitaceae, Calamitaceae, Equisetaceae

6. Phylum PTEROPSIDA Jeffrey (1899), emend. Scott (1909)
   A. Class Filicinae
      A1. Subclass Primofilices
         1. Order Protopteridiales—Protopteridiaceae
         2. Order Cladoxylales—Cladoxylaceae
         3. Order Zygopteridales (Coenopteridales)—Stauropteridaceae, Zygopteridaceae, Botryopteridaceae, Anachoropteridaceae
         4. Order Archaeopteridales—Archaeopteridaceae
      A2. Subclass Ophioglossinae
         1. Order Ophioglossales—Ophioglossaceae
      A3. Subclass Noeggerathiinae
         1. Order Noeggerathiales—Noeggerathiaceae
      A4. Subclass Marattiinae
         1. Order Marattiales—Angiopteridaceae, Marattiaceae
      A5. Subclass Leptofilices
         1. Order Filicales (Polypodiales)—Osmundaceae, Schizaeaceae, Gleicheniaceae, Matoniaceae, Loxsomaceae, Hymenophyllaceae, Hymenophyllopsidaceae, Plagiogyriaceae, Dicksoniaceae, Cyatheaceae, Pteridaceae, Ceratopteridaceae (Parkeriaceae), Aspidiaceae (Aspleniaceae), Polypodiaceae
         2. Order Marsileales—Marsileaceae
         3. Order Salviniales—Salviniaceae, Azollaceae

B. Class Gymnospermae
  B1. Subclass Pteridosperminae
    1. Order Cycadofilicales—Lyginopteridaceae, Calamopityaceae, Medullosaceae, Peltaspermaceae, Corystospermaceae, Caytoniaceae, Glossopteridaceae (?)
  B2. Subclass Phyllosperminae
    1. Order Cycadales—Cycadaceae
    2. Order Bennettitales (Cycadeoidales)—Williamsoniaceae, Bennettitaceae (Cycadeoidaceae)
  B3. Subclass Stachyosperminae
    1. Order Cordaitales—Pityaceae, Poroxylaceae, Cordaitaceae
    2. Order Ginkgoales—Ginkgoaceae
    3. Order Coniferales—Voltziaceae, Araucariaceae, Podocarpaceae, Cephalotaxaceae, Taxaceae, Pinaceae, Sciadopityaceae, Taxodiaceae, Cupressaceae
  B4. Subclass Chlamydosperminae
    1. Order Ephedrales—Ephedraceae
    2. Order Welwitschiales—Welwitschiaceae
    3. Order Gnetales—Gnetaceae
C. Class Angiospermae
  C1. Subclass Dicotyledones
  C2. Subclass Monocotyledones

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PHYSIOLOGICAL STUDIES ON THE ACTINOMYCETES [1]

D. PERLMAN [2]

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INTRODUCTION

As a result of studies on the therapeutic value of certain antibiotic metabolites produced by actinomycetes, this group of microorganisms has received intensive study during the past five years. While the major interest has been in investigating both old and new species for the production of new and potentially useful antibiotics, some aspects of the physiology of several of the antibiotic-producing actinomycetes have been elucidated as a by-product of these studies on antibiotic production. In studying these reports it appears that some of the organisms have similar mechanisms of metabolizing carbohydrates and other compounds, and these will be mentioned in the following discussion. In general, reference will be made to papers appearing after 1940. While several interesting contributions appeared prior to that date, the changes in cultural and analytical techniques during the past decade are such that it is difficult to coordinate the earlier studies with those of more recent origin. This review will not include mention of all papers that have appeared on this subject, but will be more of a summary of those which seem to be of major interest. For further

[1] This review is one of a series surveying the literature dealing with aspects of microbiological processes of interest to the fermentation industry. It is an extention of an earlier summary (409*a*), and is intended as a section of a source book. This survey covers the literature published before 1952.

[2] Present address: 35 Edgehill St., Princeton, N. J.

information the reader may wish to consult the original papers and the monographs which have appeared on some of the topics (483, 484, 515).

The pre-occupation of many investigators with the single aspect of antibiotic production has perhaps led to a rather one-sided understanding of this group of microorganisms. The actinomycetes are a rather extraordinary group and carry out many interesting biochemical reactions in addition to the production of antibiotics. Perhaps interest in these other attributes will increase either as a by-product of the antibiotic investigations or for other reasons.

PROBLEMS IN STUDYING ACTINOMYCETE GROWTH AND METABOLISM

The actinomycetes comprise a group of branching unicellular organisms essentially mesophilic and aerobic in their requirements for growth and resembling both bacteria and fungi in these respects. Waksman's recent monograph (484) discusses in detail the response of several of the actinomycetes to various environmental conditions and their importance as units of the plant kingdom. Further illustrations of the rather broad limits under which several of the species will grow are found in descriptions of processes used in or considered for production of certain antibiotics (94, 107, 398, 500, 501). Many of the recent investigations have made use of the submerged culture technique so widely used in the cultivation of fungi (334, 350, 474), rather than the older methods of growing the cultures on the surfaces of nutrient media. It appears that in general the same metabolic products are obtained when the cultures are grown under either submerged or surface culture, though the rates of metabolism of the substrates appear to be quite different, being significantly higher when the submerged culture process is used (530, 532). It is also probable that accumulation of certain intermediate metabolites occur when less oxidative environmental conditions exist, while under other conditions, e.g., introduction of more air into the growing culture or incubation at higher temperatures, these metabolites are dissimilated rapidly and may not be found in significant quantities.

The concept that a growing culture consists of a mixed population of individuals of widely divergent properties has been accepted for many microorganisms and in general seems to apply to the actinomycetes. This is perhaps substantiated by the studies on the

properties of bacteriophage-resistant strains isolated from largely bacteriophage-sensitive cultures, as well as the properties of non-antibiotic-producing substrains isolated from antibiotic-producing cultures (100, 378, 381, 492, 503). This concept of heterogeneity might be used to explain the differences in results obtained when the "same" culture is studied under what appear to be similar conditions in two different laboratories, and the differences that at times occur when the "same" culture is studied in the same laboratory at different intervals. The importance of maintenance of culture characteristics, i.e., maintenance of the culture under conditions permitting a minimum of natural variation, as well as the importance of the steps in the preparation of inoculum for experiments have apparently not been widely recognized. The literature on the use of certain actinomycetes in large-scale production of certain antibiotics suggests that the problem of stabilization of certain properties of the cultures has become of primary importance (311, 347, 474), and, although many of these reports reflect past instead of current practices, they serve to indicate the change in thought on this matter. The bacterial geneticist is as important a member of the group preparing a process for large-scale production as the microbiologist, the biochemist or the bio-engineer. A consistent fermentation performance is essential from a commercial viewpoint (474), and variations arising from changes in culture preparation, as a response to environmental or nutritional factors, must be minimized if possible.

Thus, while it is true that many actinomycetes will apparently grow well under a variety of conditions, cellular growth is not to be taken as a final end-point, since metabolic activity may vary widely under quite similar environmental conditions. Also, the chemical composition of the actinomycete cells changes in some degree with the cultural conditions and the medium (409), a further indication that growth in itself should not be accepted as an indication that similar results have been obtained under significantly different cultural conditions. Survey of the literature suggests that as the broad limits of actinomycete activities have been defined, the future studies should utilize controlled conditions and more specific techniques rather than the somewhat crude screening procedures acceptable for the exploratory studies.

PRODUCTION OF ANTIBIOTIC SUBSTANCES

INTRODUCTION. The past decade has seen considerable interest in the actinomycetes as producers of antibiotic substances. The successful use in chemotherapy of streptomycin, chloramphenicol (Chloromycetin is the trade name for this substance), aureomycin and terramycin, all metabolites of actinomycetes, has stimulated the search for new actinomycetes and new antibiotics among the actinomycetes. The early exploratory studies by McCormack (277) and Alexopoulos and Herrick (7, 8, 9) were followed by the intensive studies by Prof. S. A. Waksman and his students (480–508*a*) which culminated in the discovery of streptomycin (383, 482) and other new and potentially useful chemotherapeutic agents. Nearly 100 antibiotic substances have been reported in the literature as metabolites of actinomycetes. A few of these have been isolated in pure form and their chemistry studied in detail, while others have been described only as concentrates or in such a preliminary way that it is not certain whether a single entity or a group of substances was under study. Some of those whose identity seems firmly established are listed in Table I. Only one, chloramphenicol, has been sythesized chemically, and chemical structures have been proposed for only a few others, including streptomycin and the related mannosidostreptomycin and cycloheximide (Actidione is the trade name for this substance). All of those which have been purified and studied chemically have been found to be rather complex organic molecules, usually unrelated to substances previously described in the chemical literature.

The following generalizations may be drawn from the microbiological and chemical literature:

a). Several antibiotic substances unrelated chemically may be produced simultaneously by a given strain of an antibiotic-producing actinomycete. Examples are the production of streptomycin, grisein, mannosidostreptomycin, streptocin, cycloheximide and unidentified antibiotics by *Streptomyces griseus,* and streptolin and streptothricin by *S. lavendulae.* Certain strains of these species may produce all or only a few of these antibiotics.

b). Conversely, an antibiotic-producing strain is likely to produce several chemically related antibiotic substances. Examples of this capability are the production of mannosidostreptomycin and

TABLE I

SOME ANTIBIOTIC SUBSTANCES PRODUCED BY ACTINOMYCETES

Name	References*	
	Microbiological studies	Chemical studies
Actinomycetin	511, 513, 515	511
Actinomycin A	216, 295, 375, 437, 473a, 486, 490, 494, 512, 513, 514	77, 78, 236, 261, 295, 375a, 379, 445, 487
Actinomycin C		36, 39, 41
Actinorhodin		40
Antimycin A, B, C	259, 260	102
Aureomycin	92, 94, 351	43, 94
Aureothricin	456	270, 456
Borrelidin	30	30
Chloramphenicol (Chloromycetin)	105, 107, 146, 299, 303, 397, 447, 449, 459, 460	23, 24, 105, 246, 360, 457, 454, 459, 460, 473
Cycloheximide (Actidione)	518, 519, 520	124, 239, 240, 256
Dextromycin	298	298
Endomycin	151	151
Flavomycin	3, 10a, 171	3
Fradicin	412, 413	170, 413
Fungicidin	164, 388	45, 164
Grisein	138, 365, 366, 471	246, 365, 366
Griseolutein	300, 472	472
Litmocidin	139	139
Luteomycin	160	160, 161
Masurin	11	11
Micromonosporin	498	
The neomycins:		
Neomycin A	412, 505, 506, 338	245, 325, 326, 412, 415
Neomycin B	338	103, 257, 258, 361, 415
Neomycin C	338	103
Streptothricin BI	291, 422, 461, 462	462
Vinactins A, B, C	62, 275	62, 435
Netropsin	120, 121	120, 121
Picromycin		37, 42
Proactinomycins A, B, C	134, 271	271, 272
Resistomycin		42a
Reticulin	179	179, 181c
Rhodomycetin	390	
Rhodomycin		38, 42b
Rimocidin	31a	31a
Roseochromogen	190, 221	190
Roseomycin	290, 291	291
Streptocin	247, 504	

TABLE I (*continued*)

Name	References*	
	Microbiological studies	Chemical studies
The streptomycins:		
Streptomycin	1, 4, 262, 372, 383, 385, 493, 500	50, 127, 482, 476
Mannosidostreptomycin		128, 129
Hydroxystreptomycin	27, 408	153, 408
The streptothricins:		
Streptothricin	126, 422, 488, 490, 501	52, 53, 476
Streptin	283, 534	283, 534
Streptolin	369	369
Lavendulin	214, 215	204
Actinorubin	214, 215	204
(unnamed)	34, 187, 436, 507	34, 436
Sulfactin		204
Terramycin	363, 398	119, 322, 323, 363, 398
Thiolutin	424	424
Viomycin	108, 122, 176, 122	25, 122, 159
Xanthomycins A, B	434	181 f, 288f, 434
Unnamed acids	31	31
Unnamed anti-phlei factor	314	314

*Only representative references are included in this table. A more complete listing may be found in references 123, 483, 484.

streptomycin by *S. griseus;* neomycins A, B and C by *S. fradiae;* vinactins A, B and C by *S. vinaceus;* and xanthomycins A and B by an unidentified actinomycete. In addition, several antibiotic substances, including viomycin, streptothricin and streptolin, which have apparently quite different bacteriological properties, appear to contain a chemical entity in common.

c). Cultures producing the same antibiotics or groups of antibiotic substances are likely to be found in soil samples or other natural materials gathered from widely separated localities. At one time it was thought that cultures producing a specific antibiotic substance were not widely dispersed and that only a single culture or a few cultures existed (490, 493, 496). More recent observations which report the isolation of streptomycin-producing actinomycetes, chloramphenicol-producing actinomycetes, hydroxystreptomycin-producing actinomycetes and actinomycin-producing actinomycetes, to mention only a few, have dispelled this original

hypothesis (see Table I). In many cases these cultures producing the same antibiotic substance have far different morphological and physical characteristics, and it is apparent that morphological relationships or lack of morphological relationships may not be used in identifying an antibiotic substance. This concept of the widespread occurrence of cultures producing specific antibiotic substances has apparently not been generally accepted in the past and seems to have led to some confusion in the literature. For example, laboratories working independently reported in a preliminary way what may turn out to be the same antibiotic substance and called it streptothricin BI, neomycin and vinactin. Perhaps with wider circulation of literature and reduction in the number of preliminary reports such confusion will be reduced.

ANTIBIOTIC ASSAY METHODS. It is necessary to have practical techniques for assaying fermentation samples before progress may be made in studying antibiotic production by actinomycetes. Among the more widely used methods based entirely on responses of sensitive bacteria are the dilution test using either agar or liquid media (89, 126, 202, 212, 213, 354, 415, 491), the agar diffusion test or cup test (47, 80, 81, 109, 131, 265, 262, 358, 412, 519, 531) and the linear diffusion test (79, 274, 288*b*). Some of these procedures, notably the cup test, have been studied in the bioassay of penicillin-containing solutions (123, 165) where the antibacterial agent is a highly bacteriostatic acidic substance of relatively low molecular weight. Most of the actinomycete antibiotics are of higher molecular weight and are of neutral or basic character. They diffuse through agar at varying rates, depending in part on the pH and the composition of the agar medium (90, 126, 288*c*, 288*d*, 288*e*, 422). It has been difficult to interpret agar diffusion assays of solutions containing mixtures of antibiotic substances, some of which diffuse through the agar at faster rates than others (109, 145, 415). In these cases the dilution method has been more useful than the agar-diffusion test. Selection of the test organism for use in microbiological assays is of considerable importance, as certain bacteria have been found to be considerably more sensitive to certain antibiotic substances than to others (483). In some instances some success has been obtained in using antibiotic-resistant or antibiotic-dependent test organisms (112, 193, 248, 251, 356, 484, 477, 491), although these organisms may not be entirely

satisfactory because of the non-specificity of antibiotic resistance (112, 118, 319) and antibiotic dependency (82, 374).

As the chemistry of the actinomycete antibiotics has unfolded, several wholly or partly chemical procedures have been proposed for the analysis of fermentation samples. In the case of streptomycin these have been based on the formation of maltol from streptomycin by alkaline degradation (110, 389), and in mannosidostreptomycin on the formation of hydroxymethylfurfural by acid degradation (114, 330). While these methods are reasonably satisfactory (374*a*) when analyzing aqueous solutions of the pure antibiotic materials, analysis of fermentation samples containing complex organic substances often leads to complications and uncertainty. The use of filter-paper partition chromatography (177, 181*d*, 191, 192*a*, 407, 408, 418, 524) to indicate qualitatively and quantitatively the antibiotics present in fermentations has not been wholly satisfactory, perhaps becouse of interferences by inorganic ions (341) and other substances. While mixtures of relatively pure actinomycete antibiotics by the liquid-liquid counter-current extraction technique have been analyzed satisfactorily (271, 272, 310, 346, 414, 415), application of this elegant technique to crude materials, such as fermentation samples, has apparently not been feasible, perhaps due to emulsion formation. It appears that, while the chemical assay methods are to be preferred because of their specificity, the microbiological methods have been used and probably will continue in favor in the future, as they are more sensitive and, if controlled properly (47, 274), perhaps just as accurate as the chemical methods.

SELECTION OF ACTINOMYCETE CULTURES. Many papers have appeared during the past few years, directly or indirectly illustrating techniques useful in isolation of actinomycetes from natural habitats and surveying the antibiotic-producing abilities of these actinomycetes (2, 48, 55, 92, 93, 95, 123, 198, 199, 208, 254, 388, 439, 483, 484, 489). In some of these special media have been used which encourage the growth of the actinomycetes over the other microorganisms present in soil samples (48, 95, 93, 123, 254, 292, 294, 387, 388, 483, 428, 484). In certain instances small quantities of specific antibiotic substances have been added to media as a result of the observation that streptomycin-producing actinomycetes are more risistant to streptomycin than non-strepto-

mycin-producing actinomycetes (*253b*, *253c*, 281, 446, 451, 483, 484, 497) and that chloramphenicol-producing actinomycetes are more resistant to chloramphenicol than non-chloramphenicol-producing actinomycetes (449, 457). Efforts have been made to identify the antibiotic substances produced by newly isolated actinomycetes as quickly as possible after isolation so that time will not be spent in studying the production of undesired antibiotic substances. Reactions of antibiotic-dependent bacteria (193, 356, 477), antibiotic-resistant bacteria (112, 248, 249, 251, *253a*, 446, 448, 451, 484, 491) and bacteriophage concentrates (499) are a few of the techniques used in these screening tests. The interpretation of the results of these preliminary studies are too often complicated by the actinomycete under study producing more than one antibiotic substance (340, 436), the lack of specificity of the antibiotic-resistant and antibiotic-dependent bacteria (82, 112, 141, 319), and the considerable sensitivity of certain actinomycetes to certain actinomycete antibiotics which might be contaminating the bacteriophage preparations used (106), as well as natural resistance of the actinomycete to the bacteriophage. Identification of antibiotic-producing actinomycetes purely on the basis of morphology (6, 32, 75, 76, *76a*, 200, 201, 244, 251, 305, 308, 353, 450) or such physiological characteristics as nitrate reduction (64, 140), pigment production (68, 251, 315, 450) or ability to utilize certain carbohydrates (*247a*, 305, 304, 308, 352, 471) have been found to be rather unsatisfactory procedures. Many morphologically and physiologically different actinomycetes apparently produce the same antibiotic substances, while morphologically similar cultures may not produce the same antibiotic substances (51, 55, 138, 244, 251, 303, 305, 308, 365, 366, 497).

After the antibiotic-producing actinomycete has been selected for further study, it has been a common practice to attempt to increase the yield of the desired antibiotic substance by selection of higher-producing cultures or more suitable media and fermentation conditions. In the first of these procedures this has been accomplished by selection of substrains derived from the original culture by various procedures. These have included, in addition to study of the natural variation (17, 100, *101a*, 209, 244, *299a*, 381, 382, 384, 402, 434, 492, 519), induction of mutations by exposure to ultraviolet light (17, 93, 100, *101a*, 306, 345, 381, 402, 519, 540) and x-rays (17, *101a*, 307, 381), exposure of the actinomycete

spores to nitrogen-mustard compounds (17, 100, 306, 345) and exposure of the spores to antibiotics and other chemicals (17, 100, 133, 281, 288, 288*a*). Marked success has been obtained in many instances by the exposure of the spores to ultraviolet light and x-rays; strains surviving these treatments often produce several times as much of the antibiotic as the original culture (17, 93, 100, 307, 381, 402, 519, 540). The use of ultraviolet light is complicated by the photoreactivation phenomenon (217, 219). Since many antibiotic-producing actinomycetes are susceptible to bacteriophage infection, the selection procedures (237, 364, 396, 474, 536) used in obtaining bacteriophage-resistant strains may result in selection of strains producing more or less of the desired antibiotic. In several instances it has been found that strains isolated from antibiotic-producing actinomycete cultures by these different procedures differ from the parent culture in that they produce only a single antibiotic substance, while the parent culture produces a mixture (381, 519). It has also been possible to select substrains producing more of one component of a mixture of antibiotic substances than the parent culture (330, 381, 519). There are reports (216, 218) that exposure of spores of non-antibiotic-producing cultures to ultraviolet light has resulted in mutations, some of which produce antibiotic substances. A change in morphological and physiological characteristics often accompanies this change in antibiotic-production capacity induced by the above selective procedures (100, 101, 345, 381).

MEDIA USED IN PRODUCTION OF ANTIBIOTIC SUBSTANCES BY ACTINOMYCETES. A considerable number of media have been used in studying the antibiotic-producing actinomycetes, each laboratory apparently favoring certain compositions over others. The formulations of a few of these media have been summarized by Waksman and others (113, 123, 381, 483, 484, 503). Many of these media have contained materials of natural origin, such as soybean meal and other seed meals (31, 91, 107, 120, 121, 355, 369, 397, 430, 433*a*, 438, 468, 482), peptones and other digests (28, 28*a*, 66, 94, 107, 151, 146, 280, 317, 372, 397, 484), as well as yeast and other microbial products such as distiller's solubles (66, 67, 278, 262, 279, 280, 284, 285, 286, 397, 431). These nitrogenous materials have been used as supplements or as the main ingredients of the media. No reports appear to be available to indicate that these

complex nitrogenous materials provide specific precursors for the production of certain antibiotic substances. However, there are several observations (178, 210*a*, 309, 381, 481, 503) that certain strains produce more antibiotic-substances on one medium than when grown on another medium, and a "poor" producing culture on one medium may be an "excellent" culture on another medium. Perhaps the main advantage in the use of such complex media lies in the rapid growth of the actinomycete obtained when these media are inoculated with spores or vegetative growth.

Many of these complex nitrogenous supplements contain considerable quantities of inorganic material, and in certain fermentations this inorganic material has been found to affect the antibiotic production significantly (15*a*, 58, 59, 62, 275, 317, 359, 401, 429, 431, 506, 535), in some instances shifting the fermentation from the production of one type of antibiotic substance to others (275).

The hypothesis that certain substances of natural origin are needed for antibiotic production (28, 460, 490, 500) is no longer tenable, for antibiotic-producing actinomycetes have been grown on synthetic media with moderately high rates of antibiotic production (98, 99, 148, 293*a*, 338, 379, 432, 433). Several of these synthetic media have contained carbohydrate, inorganic nitrogen source and inorganic salts (19, 98, 99, 149, 293*a*, 432, 433). However, there have been several suggestions that the addition of certain supplements which might act as precursors for the production of specific antibiotic substances (chloramphenicol) have been sometimes successful (147, 460), suggesting some of the biosynthetic steps in the production of these antibiotics. As most actinomycetes will utilize a number of carbohydrates (94, 99, 183, 369, 379) and lipids (332, 336) as energy sources, and many are able to utilize ammonia or nitrates as nitrogen sources (11, 64, 65, 369, 379, 432), considerable latitude may be found in the formulation of synthetic media. The advantages of using a synthetic medium in studying antibiotic production by actinomycetes appear to lie in the knowledge of its exact composition, its reproducibility and usually in its simplicity. The disadvantages are apparent when the yields of antibiotic substances and other metabolic products obtained when the actinomycete is grown of the synthetic medium are lower or different than when the culture is grown on media containing natural materials.

LARGE-SCALE PRODUCTION OF ACTINOMYCETE ANTIBIOTICS. The stages in the development of the commercial processes for the production of streptomycin (123, 350, 474, 482, 493), chloramphenicol (107, 299*a*, 311, 350, 540*b*) and terramycin (360*a*) have been described by several observers. It seems probable from the fragmentary information available that the equipment and practices used in the production of aureomycin (94) and terramycin (360*a*, 398) are similar in many respects to those used for the production of streptomycin. While at one time some streptomycin was produced by the surface culture process (1, 493), the success and advantages of the submerged culture process have been such that the latter technique is the only one used now. The production of these actinomycete antibiotics has reached a tremendous scale: in the United States alone that of streptomycin in 1951 was nearly 125 tons, and production plants were planned or in operation in eight other countries (393, 474).

The processes used in the production of actinomycete antibiotics may be divided into those having to do with the fermentation phase and those dealing with the recovery phase. These two phases are quite interdependent, and a change in a fermentation stage may affect the recovery operations quite markedly, while changes in the recovery processes may necessitate changes in the fermentation operations. It is not at all unusual that proposals for increasing the production of an antibiotic substance by changing the fermentation process by the use of new media or a new strain must undergo considerable study from the recovery process viewpoint before they may be put into practice.

The fermentation processes used in actinomycete fermentations resemble in general those used for the production of penicillin (123, 125, 320, 334, 350), and since they chronologically followed the development of penicillin production processes, they have taken over some of these earlier tested practices. The cultures are started from spore stocks maintained on soil (311, 347, 410, 474, 484, 493) or other menstra (238, 299*a*, 410, 474), and the culture is grown vegetatively in shaken flasks and then transferred to aerated units until sufficient growth is obtained to inoculate the final fermentation stage (57*a*, 402, 474, 482, 493, 539). The actinomycetes tend to form " submerged " spores (54, 56, 174, 484)

when grown under submerged conditions and the mycelium tends to fragment as it ages (54, 484), a condition which limits the length of the fermentation cycles in certain instances. Repeated transfers of *S. griseus* cultures at frequent intervals in the vegetative state are said to result in cultures which have lost significant antibiotic-producing capacity (466). Continuous and semi-continuous fermentation processes have been proposed (86), but apparently many plants have not been able to take advantage of these techniques.

As in all aerobic fermentation processes, considerable quantities of sterile air are required for these fermentations, and many procedures have been suggested for sterilizing this air (222, 223, 264, 282, 289, 321, 318, 320, 404, 427). Most production units apparently use fermentors which are mechanically agitated as well as aerated (67, 86, 116, 123, 311, 347, 420, 464, 474, 482). As agitation and aeration are quite costly operations, a number of laboratory-size and pilot-plant units have been proposed to study problems involved in obtaining maximum efficiency in power utilization. The laboratory-size units also permit evaluation of certain fermentor designs and construction materials (46, 125, 184, 241, 269, 370, 405, 417, 420, 421, 464, 470). The studies so far reported have not entirely solved the problems involved, and it is apparent that considerable quantities of the air introduced into the fermentors are not utilized directly by the actinomycetes (20, 21, 130, 132, 220, 468, 474, 526, 527, 528). Also, sometimes a strain is developed which apparently is less tolerant of certain metallic ions as far as antibiotic production is concerned, or requires more or less air than other cultures, both peculiarities affecting fermentor operations (15*a*, 421, 464).

In common with many other fermentation operations, such as the production of butyl alcohol, sorbose and penicillin, it is essential to conduct the fermentation in such a manner that all foreign microorganisms are excluded if efficient operation of the plant is to be obtained (195, 474). Several bacteria are capable of destroying some of the actinomycete antibiotics, including streptomycin (12, 349) and chloramphenicol (140, 395), and, although they are not so effective as penicillinase-producing organisms are in reducing the yields of penicillin (123, 334, 533), they do affect the fermentation performance as well as the recovery operations. Actinomycetes may also be infected with bacteriophages (5, 28, 87, 106,

142, 211, 237, 242, 243, 300*a*, 300*b*, 364, 377, 396, 465, 474, 484, 521, 522, 536) which very often results in complete interruption of the fermentation process. This effect may be minimized by certain procedures (186, 325, 474), but these measures are not a replacement for the operation of the fermentation in the absence of the contaminating bacteria and bacteriophage infection. As in other fermentations liable to bacteriophage infection, the use of bacteriophage-resistant cultures is essential for consistent uninterrupted operation (335, 474).

The length of the fermentation period is usually adjusted to yield the maximum quantity of antibiotic substance per unit time of operation (347, 474), and the composition of the fermentation medium is so adjusted that only the needed quantities of ingredients are present. At the conclusion of the fermentation period the mycelium and suspended solids are removed by filtration or other means. Addition of acid to the whole culture before filtration has facilitated filtration as well as released the antibiotic bound to the mycelium (331, 357). These advantages resulting from acidification before filtration were first observation in the streptomycin process (357), and have been applied more recently to other actinomycete fermentations (122, 258, 363, 398).

The antibiotics present in this acid-filtrate may be recovered by a number of processes, depending on their chemistry. Streptomycin and other basic antibiotics may be removed from the filtrate by carbon adsorption (50, 187, 262, 367, 412, 476, 482, 500, 539), adsorption on ion exchange materials (182, 188, 235, 258, 412, 426, 476, 479, 543) or by solvent extraction using carrier systems (310, 366*a*, 469, 473*a*). The basic antibiotics may then be further purified by chromatography (33, 50, 103, 128, 258, 324, 327, 347, 482), precipitation by certain reagents (50, 127, 360*a*, 362, 453, 482, 525, 542) and/or by the formation of certain complexes (266, 482). Somewhat different processes have been used in the recovery of chloramphenicol (23, 24, 105, 107, 454, 460), the actinomycins (36, 37, 236, 261), borrelidin (30), cycloheximide (124, 518), terramycin (363, 398) and aureomycin (94), in general taking advantage of the possibilities of purifying these antibiotic substances by extracting them from the filtrate with solvents. Very often the recovery processes are considerably more complicated than the fermentation operations, involving many more operations and techniques. A discussion of these problems is outside the

scope of this review, and for more information reference may be made to some of the recent literature (123, 350, 482, 474).

Disposal of the fermentation medium solids and of the "spent" filtrate is a considerable problem and has recently become of primary importance in the operation of production units. As much as 600 pounds of solids and 20,000 gallons of liquid may be the residues from the production of 50 kilograms of streptomycin. Incineration as well as biological disposal units have been successfully operated to dispose of this waste material. The solids contain a significant amount of proteinaceous material (116, 409) as well as some residual antibiotic substance and growth factors (181*a*), and there has been some demand for this residue as a feed supplement for animals and poultry (116, 474).

THE RÔLE OF ANTIBIOTIC BIOSYNTHESIS IN ACTINOMYCETE METABOLISM. The rôle of antibiotic biosynthesis in actinomycete metabolism must be considered in studying the production of antibiotic substances by actinomycetes. While the analyses of the cells of *S. griseus* suggest that the amino acids do not differ in quantity from those in other microorganisms (409), the observation that a substantial quantity of streptomycin (usually more than half of that produced) occurs bound to the mycelium (331, 334, 357) suggests that perhaps the antibiotic substance is a part of the cell wall of the actinomycete. This bound streptomycin may be released, as indicated in Table II, by treatment of the cells with acid, alkali or ionizable salts (331), but not by disintegration of the cell by various means, including sonic energy (293*a*), bacteriophage (335, 465) and enzymatic treatment (328). Significant quantities of other actinomycete antibiotics, including streptothricin (331), the neomycins (331), chloramphenicol (331), aureomycin (94, 331) and unnamed substances (31), have been found to occur bound to the mycelium of the respective actinomycetes and may be released by treatment with acid, alkali or ionizable salts. This binding does not appear to be a simple ion-exchange phenomenon, since addition of streptomycin to a mycelium sample does not result in adsorption (331), and the "binding" power of the mycelium is apparently not a function of the weight of the mycelium.

While the streptomycins (26, 50, 129, 153, 407, 408, 482), the streptothricins (52, 53), the neomycins (103, 245, 258, 325, 326, 361), viomycin (25, 122, 159) and streptolin (53) may be consid-

TABLE II

RELEASE OF ANTIBIOTIC ACTIVITY FROM MYCELIUM OF *Streptomyces griseus* BY CHEMICAL AND PHYSICAL TREATMENTS

Treatment of mycelium suspension*		Antibiotic potency released by treatment
		μg./ml.
None		19
Heated in boiling water bath for 10 minutes		38
Exposure to sonic energy for 15 minutes		107
Addition of sufficient concentrated HCl to give:	pH 5.1	37
	pH 4.2	67
	pH 3.0	109
	pH 2.5	188
	pH 1.8	175
Addition of sufficient 10*N* NaOH to give:	pH 8.0	45
	pH 9.1	109
	pH 9.9	161
	pH 10.8	143
Addition of sodium chloride to give conc.:	0.003 M	19
	0.01	19
	0.03	33
	0.1	53
	0.3	97
Addition of sodium sulfate to give conc.:	0.003 M	19
	0.01	43
	0.03	97
	0.1	159
	0.3	147
Addition of sodium citrate to give conc.:	0.003 M	25
	0.01	64
	0.03	142
	0.1	129
	0.3	130

*Samples from four-day-old fermentation were centrifuged and the supernatant liquid assayed. There were 108 μg./ml. of streptomycin in the supernatant liquid. The collected solids were resuspended in distilled water to original volume, and acid, alkali, or salt was added as indicated to ten-ml. aliquots. After 15 min. shaking on a mechanical shaker the solids were collected by centrifugation and the supernatant liquid submitted for assay. Abstracted from (331).

ered to be polysaccharides, perhaps related to the cell-wall polysaccharides of microorganisms, other actinomycete antibiotics, including chloramphenicol (23, 69, 105, 360) and the actinomycins (36, 39, 41, 77, 78), do not apparently have this structure and in general may be thought to be polypeptides. As several of the antibiotics have common chemical moieties (streptothricin, viomycin, streptolin (52, 53) or similar ones (the streptomycins and neomycins (103, 153, 326, 482, 408), they might be formed by a somewhat similar chain of enzymatic reactions. This hypothesis does not help to explain the differences in antibiotic spectra between the apparently chemically similar antibiotics, nor does it elucidate the mechanism of sensitivity of *S. griseus* strains to neomycin (506) and the sensitivity of non-streptomycin-producing cultures to streptomycin (496).

This sensitivity of actinomycete cultures to antibiotic substances produced by other actinomycete cultures has been used as a method of selecting and identifying actinomycete cultures (163, 251, 252). Apparently, while a strain is sensitive to its own antibiotic to some extent, it is much more sensitive to other antibiotic substances. Certain strains of *S. griseus* have been found to be much less sensitive to streptomycin than most cultures (301, 523) of *S. griseus*, and exposure to streptomycin has been used as a method of selection of new cultures (32, 281), presumably higher streptomycin producers (101, 281). A similar procedure has been used in selection of chloramphenicol-producing cultures (457). In their first experiments Schatz and Waksman (383, 385, 500) included 0.5% sodium chloride in their media. Later studies, similar to those summarized in Table II (13, 111, 331), suggested that this salt component of the medium acted to free the "bound" streptomycin and that recognizing the sensitivity of the actinomycete to streptomycin, higher antibiotic production might be obtained when the salt was omitted from the medium. This hypothesis was proved by several experiments (331) with results similar to those in Fig. 1. It seems probable that omission of the salt component of media used in the production of other antibiotic substances will also result in increased yields.

Calculation of the efficiency of substrate conversion to streptomycin indicates that in some instances approximately 15% of the carbon added to the medium, either as glucose (98, 101) or glycerides (332, 336), may be converted to streptomycin, and probably

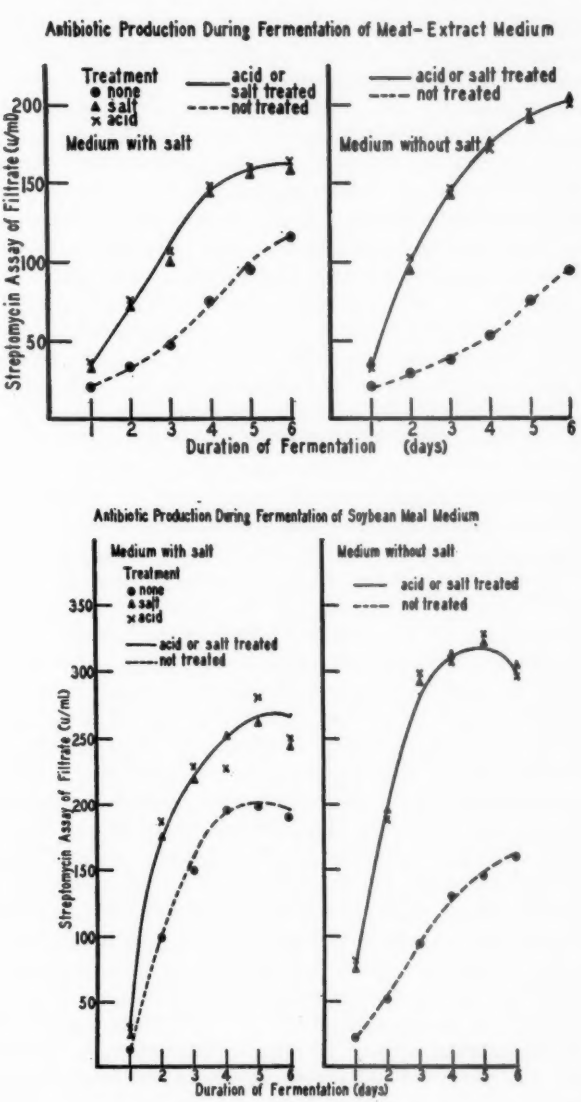

FIG. 1. Studies on the effect of sodium chloride on antibiotic production by *Streptomyces griseus* (Waksman strain # 4). *Upper*: soybean meal-glucose medium; *lower*: meat extract-peptone-glucose medium. Abstracted from (331).

similar efficiencies of conversion may be obtained with other actinomycetes. This formation of a polysaccharide molecule from saturated fatty acids or glycerides is truly an exhibition of the amazing synthetic powers of the actinomycetes. The actinomycetes producing aureomycin (343) and chloramphenicol (316) are capable of incorporating substantial quantities of the chloride ion present in the medium into the antibiotics, and it is probable that the grisein-producing strains of *S. griseus* do likewise with iron (246).

Considerable time and effort have been expended investigating some of the chemical changes that occur when antibiotic-producing actinomycetes ferment the medium, in the hope that some light might be shed on the mechanisms of antibiotic formation and the optimum conditions for antibiotic production. Among the organisms studied are *S. griseus* (97, 136, 144, 145, 176*a*, 328, 336, 342, 378, 481, 495, 537), *S. venezuelae* and other chloromycetin-producing cultures (316, 540, 540*a*), and the actinomycetes producing streptolin (369) and the xanthomycins (434). It does not appear that these investigations have shed much light on the mechanism of antibiotic production, since the same changes seem to occur in antibiotic-producing as well as non-antibiotic-producing strains (328). Highest antibiotic production seems to be obtained when the metabolism of the actinomycete is so regulated that the pH of the growing culture is maintained between pH 7 and pH 8. This regulation may be obtained by suitable adjustment of the medium with acid or alkali directly, or indirectly by limiting the ammonia production or acid production. This latter control is effected by adjusting the formulation of the medium with regard to the nitrogen source, or buffering the medium with suitable agents, e.g., potassium phosphate or calcium carbonate. Addition of inorganic phosphate to *S. griseus* and *S. fradiae* fermentations has been found to speed the utilization of carbohydrates (336) and in effect shorten the period that the fermentation is maintained between the desired pH limits. When slowly fermented carbohydrates are substituted for glucose, this pH control may be maintained with less difficulty. Some of the carbohydrates used for this purpose are lactose (369), starch, dextrin or maltose (506) and sucrose (31). Use of lipids as energy sources also seems to result in more satisfactory pH control (336). In spite of these extensive studies it appears that there are still problems to be solved in studying the question of finding the optimal conditions for antibiotic production.

While the sensitivity of the actinomycete to the antibiotic substances it produces must be included among the factors limiting antibiotic production, in certain instances, including the cultures producing streptothricin (501, 530, 532) and aureomycin (94), the actinomycete seems capable of apparently decomposing or inactivating the antibiotic substance. It is not certain whether this is accomplished by direct enzymatic action in attacking the antibiotic substance or by formation of such agents as hydroxylamine which inactivate many antibiotic substances (478). Perhaps the "low" antibiotic-producing cultures produce more of these "inactivating agents" and thus "detoxify" the antibiotic substance from the actinomycetes viewpoint. Unfortunately acceptance of this detoxification hypothesis raises the question of the reason for the microorganism synthesizing the antibiotic substance. Certainly the levels of antibiotic substances found in the soil (2, 148, 150, 151*a*, 348, 349, 392) or other natural habitats are far below the levels which might be inhibitory to the majority of microorganisms of the soil. The observations (248, 249, 309, 458, 467, 507, 543) that different antibiotic substances are produced when certain actinomycetes are grown on agar media than when they are grown on liquid media, as well as those indicating that addition of inorganic salts sometimes shifts antibiotic production from one type of substance to another (275), tend to work against this "detoxification" hypothesis.

METABOLISM OF CARBOHYDRATES AND LIPIDS BY ACTINOMYCETES

The actinomycetes are capable of utilizing a large number of carbohydrates as energy sources when the carbohydrates are present in the media as sole sources of metabolizable carbon, and even a large number when combinations of carbohydrates are used. While utilization of certain carbohydrates has been proposed as an aid in the taxonomic study of the actinomycetes (106, 253, 352, 353, 49), it seems possible to find such variations among a given species that the usefulness of these tests may be questioned. Some of these variations may arise from the differences in the metabolic activities of young and physiologically mature cells.

Most studies on the metabolism of carbohydrates by *S. griseus* (96, 97, 136, 137*a*, 143, 144, 145, 268, 293*a*, 328, 336, 452, 464, 495, 537) have indicated that carbon dioxide is the main metabolic product, and have not reported any accumulation of significant

quantities of intermediates in this oxidation. There is some doubt that the Krebs tricarboxylic acid cycle operates in *S. griseus* metabolism of glucose (137). The report of succinic acid production by other species indicates that at least some of the carbohydrate is metabolized by these organisms by way of a carboxylic acid cycle (63, 65*a*). While lactic acid has been reported in *S. griseus* (96, 97, 336, 384) and certain *S. lavendulae* (532, 480) fermentations, and acetic acid and ethanol in others (333), these are apparently transitory products accumulating under fermentation conditions inhibiting normal growth of the actinomycete. They are metabolized by these organisms if the fermentation period is extended or if aeration is increased (183*a*). In certain *S. fradiae* fermentations where the medium is poorly buffered, sufficient acetic acid accumulates to inhibit further growth and metabolism (506). In these cases the accumulated acetate is metabolized when the pH of the medium is raised. Propionic acid has been reported as a metabolite in *Micromonospora* fermentation (185). It is interesting to note that while media containing a large number of carbohydrates will support the growth of *Streptomyces griseus,* streptomycin production is low or absent except when glucose, starch or maltose is present (97, 99, 183). It has been reported that streptomycin containing C^{14} has been obtained when *S. griseus* was grown on media containing carbohydrate labled with C^{14} (372*a*). Also, as mentioned before, addition of inorganic phosphate to *S. griseus* fermentations results in increased rates of glucose utilization (96, 336), coincident with almost complete suppression of streptomycin production (137*a*).

Some actinomycetes produce significant quantities of extracellular amylases (96, 97, 328), while others seem lacking in these enzymes (338). The enzyme hydrolyzing mannosidostreptomycin to streptomycin is perhaps an amylase and is found in certain strains of *S. griseus* (60, 61, 255, 332). There is a report on what may be a pectinase which hydrolyzes agar (402), found in *Streptomyces coelicolor.*

There have been few attempts to determine whether the enzymes involved in carbohydrate utilization by the actinomycetes are different from those in yeast and other microorganisms. A single report suggests that cytochrome is present in certain actinomycete cells (376).

The utilization of lipids by *S. griseus* (336), *S. fradiae* (336),

S. aureofaciens (174, 175, 336) and *S. venzuelae* (336) has been recently studied. These actinomycetes appear to oxidize the lipids (glycerides or fatty acids) completely to carbon dioxide, and under the fermentation conditions used, no significant quantities of intermediate metabolites accumulated. An unidentified compound accumulated during the metabolism of glycerol by *S. venezuelae* which may have been an intermediate in the metabolism of this substrate by this actinomycete (316).

The oxidative powers of the actinomycetes have been used in partially oxidizing other substrates such as steroids. The conversion of estradiol to estrone by washed cells of *Streptomyces albus* (516), the conversion of pregnenolone to progesterone by *S. griseus* and other actinomycetes (337), and progesterone to hydroxyprogesterone (338), are examples of the use of actinomycetes for what are apparently one-step oxidations.

METABOLISM OF NITROGEN-CONTAINING SUBSTANCES

The proteolytic abilities of the actinomycetes have been studied for many years (481, 484). These organisms as a group are capable of attacking plant and animal proteins, hydrolyzing them to amino acids, polypeptides and ammonia, and have been utilized as practical and economical sources of proteolytic enzymes (84, 85, 276). As mentioned earlier, many of the media used in processes for the production of antibiotics contain such proteinaceous materials as soybean meal and other seed meals, cornsteep liquor, peptones and animal protein hydrolyzates, yeast and casein, and in growing on these media the actinomycetes produce ammonia and other hydrolytic products. The actinomycetes are also capable of utilizing nitrates and ammonia as sources of nitrogen for cellular activities (64, 65, 98, 338). Incorporation of nitrate or ammonia-nitrogen into such complex molecules as streptomycin, vitamin B_{12} or actinomycin must involve a considerable number of intermediate reactions.

Several actinomycete cultures in common with *Aspergillus* cultures are able to synthesize the interesting polypeptide, L-leucyl-L-proline anhydride (196). The synthesis of this peptide is of especial interest, as high yields of streptomycin (98, 328) and other antibiotics (338) have been obtained when proline was the source of nitrogen in synthetic media. Proline, however, does not appear to be a direct precursor of streptomycin, since certain strains of

S. griseus do not produce measurable titers of streptomycin on proline-containing or on any other media, even though normal quantities of mycelia are obtained (328). Thus, while a number of amino acids have been used in the formulation of synthetic media, including proline, glutamic acid, arginine, aspartic acid, glycine and histidine (98, 111, 328, 338), the observations that actinomycetes will utilize ammonia-nitrogen, and that some of the actinomycetes will degrade these amino acids to ammonia (328, 530), suggest that the carbon residue of the amino acids is perhaps more important in the actinomycetes economy than the nitrogen-containing groups.

PRODUCTION OF VITAMINS AND GROWTH FACTORS

The announcements that vitamin B_{12} has been isolated from actinomycete fermentations (368, 10, 263) stirred up considerable interest in possible production of this very active vitamin by microbial fermentations. Apparently nearly all actinomycetes will produce some vitamin B_{12} activity (as measured by microbiological assays) if cobalt salts are added to the media to serve as precursors (167). As cobalt is a rather effective bactericidal agent, this precursor must be added carefully (135). The fermentations producing the antibiotics streptomycin, aureomycin, grisein and neomycin will all produce some vitamin B_{12} as well if the medium is supplemented with cobalt (263, 293, 391, 380, 368, 167) without apparently effecting the yields of antibiotic substances. A survey of other actinomycetes in several laboratories has indicated that several non-antibiotic-producing cultures produce more of this vitamin than those producing antibiotics (154, 155, 156, 135, 353, 391, 380). It has also been possible by selective measures, including mutation of the actinomycetes by ultraviolet light and x-rays, to increase the yields of vitamin B_{12} produced by certain cultures (353, 380, 391). In nearly all of these studies a microbiological assay has been used, and as the chemical studies progress it is apparent that there are several forms of vitamin B_{12} (10, 126*a*, 205, 206, 207, 344), perhaps not all occurring naturally but being derived during isolation procedures. Vitamin B_{12} as it exists in nature may be produced by bacteria or actinomycetes (371). It has found use as an animal feed supplement as well as in human nutrition, and there are prospects that it will be produced by actinomycete

fermentations as a main-product as well as a by-product of antibiotic-production (154, 135, 380, 425). The incorporation of the cobalt into the actinomycete metabolite has been proved by addition of radioactive cobalt to fermentations and isolation of the vitamin containing the labled isotope (57). Actinomycetes have been reported to produce other porphyrin-like compounds (72, 73, 74) as well as grisein, an iron-containing actinomycete metabolite (246).

Some of the vitamin B_{12} produced by actinomycetes occurs, like the antibiotics, bound on the cells. It may be released by treatment of the cells with acid, alkali, ionizable salts, sonic energy or heat, and thus differs from the antibiotics in the strength of the " bonds " holding it to the cell (293*a*, 425). Subsequent to release, the cell-free liquid may be treated with cyanide to convert the vitamin present (529) to the more stable cyanide form (205).

Actinomycetes have been shown to produce other of the water-soluble vitamins (181*a*), with special studies on production of thiamin (168, 169) and the pteroylglutamic acid derivative that is active in promoting the growth of certain strains of *Leuconostoc citrovorum* (115) and coenzyme A (151*b*).

SUMMARY

Studies on the physiology of the actinomycetes have not been too extensive to date. The literature indicates that members of this group of microorganisms are able to oxidize carbohydrates and lipids to carbon dioxide, but the intermediates in this chain of reactions are unknown. Most of the actinomycetes are quite proteolytic and attack proteins and polypeptides, and are also able to utilize nitrates and ammonia as sources of nitrogen. Nearly all synthesize vitamin B_{12} when grown on media containing cobalt salts, and many are able to synthesize rather complex organic molecules which have antibiotic properties. The mechanism of synthesis of these substances is not understood, and their role in the organism's metabolism has not been investigated.

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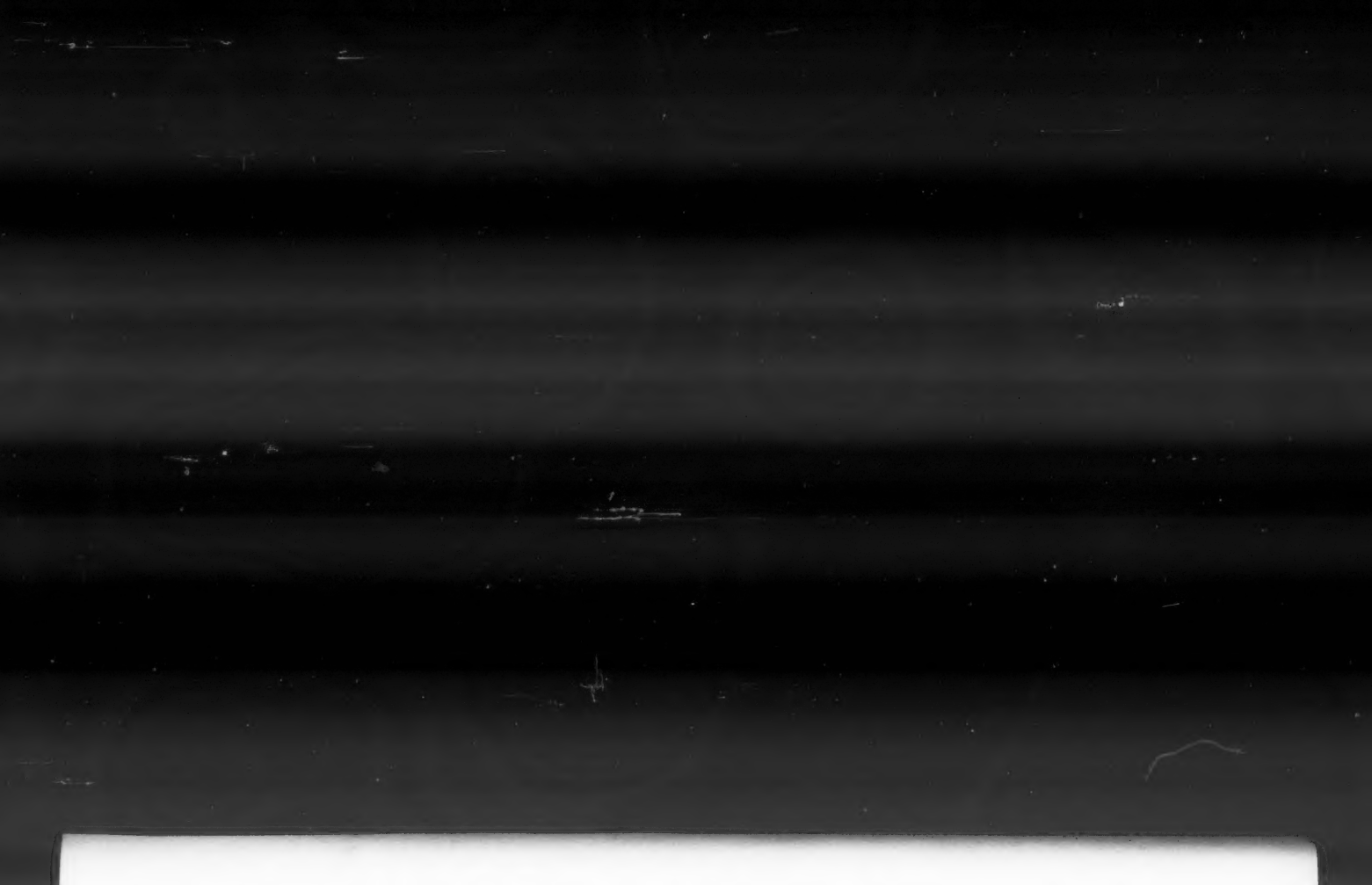
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